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FINAL REPORT P-4572/G

Application of a Newly Developed Fermenter-Purifier System for the Production of Fuel/Alcohol

for

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APPLICATION OF A NEWLY DEVELOPED FERMENTER-PURIFIER SYSTEM FOR THE PRODUCTION OF FUEL/ALCOHOL

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APPLICATION OF A NEWLY DEVELOPED FERMENTER-PURIFIER SYSTEM FOR THE PRODUCTION OF FUEL/ALCOHOL

EXECUTIVE SUMMARY

The application of a novel fermenter-purifier system to the fermentation of glucose to acetone/butanol/ethanol (ABE) by Clostridium acetobutylicum, ATCC 824 has been investigated.

The data confirm that the concept of simultaneous fermentation, removal and recovery of volatile solvents by vapour phase stripping is feasible. However, due to the more complex metabolic control mechanisms of the ABE fermentation compared with those of ethanol formation by Saccharomyces cerevisiae, additional studies are required to optimize the yield and stabilize the ABE fermentation under the conditions of continuous vapour phase stripping. Factors which have been discussed include the relationship between the onset and maintenance of solventogenesis and the organism's growth curve; nutritional requirements; the effect of the continuous vapour phase stripping on the cultural environment and hence the cells ability to produce solvents; culture morphology; and systems for the removal/recovery of entrained volatiles from the recycle gas stream. Techniques to recover highly volatile components, e.g., acetone, from the vapour phase have been investigated and impressive concentration factors for the recovery of all solvents have been noted with the use of solid phase molecular sieve adsorbants.

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Preliminary data indicate that the fermenter-purifier concept employed with the use of selective adsorbents could significantly improve the economics of the production of butanol/acetone from glucose by Cl. acetobutylicum. It is recommended that future studies to achieve steady state continuous fermentation should concentrate on the design of a gas tight fermenter system to further investigate the effect of continuous gas stripping on the metabolic control processes involved in the complex ABE fermentation.

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APPLICATION OF A NEWLY DEVELOPED FERMENTER-PURIFIER SYSTEM FOR THE PRODUCTION OF FUEL/ALCOHOL

1. INTRODUCTION

This report is submitted in accordance with the terms of DSS Contract No. OSR84-00146, DSS File No. 05SR.31926-4-5029, entitled "Application of A Newly Developed Fermenter-Purifier System for the Production of Fuel/Alcohol".

2. OBJECTIVE

The overall objective of this program has been to develop a cost effective continuous butanol fermenter-purifier system based on the gas stripping fermenter design developed at Ontario Research Foundation.

Several intermediate objectives were identified in the proposal No. P-4572/g including:

a) Selection of a micro-organism which can proliferate and ferment under the operating conditions peculiar to the process,
b) Investigation of conditions under which extended continuous operation can be carried out,
c) Demonstration of the feasibility of the concept,
d) Development of sensor/controller devices to permit unattended operation of the system
e) The determination of a mass and energy balance for the system on an extended run to permit assessment of the economic viability of the process.

3. BACKGROUND

Irrespective of the recent sharp declines in the cost of petroleum, the search continues for energy feedstocks to replace the dwindling reserves of more easily recovered and processed petroleum feedstocks. Thus, although new reserves are being identified in Canada, the location of such new sources, e.g., Arctic regions or off-shore, or the nature of the feedstock, e.g., tar-sand, make the economic recovery and processing a difficult proposition. The ultimate goal is to develop sources of fuels to supplement and extend available petroleum reserves. In Canada, particular attention is being paid to maintaining continuity of supply by using renewable feedstocks, i.e., biomass, derived from the energy of the sun. However, this requires that technology can be developed for the economic conversion of biomass feedstocks to fuel components such as methanol, ethanol, n-butanol, etc.

n-Butanol (hereafter referred to as butanol) is an attractive alternative to the lower alcohols (methanol and ethanol) as a diesel supplement. The latter alcohols are difficult to blend with diesel and under the typical winter temperatures in Canada, phase separation of the alcohol from the diesel will readily occur. Butanol has two particular attributes as a diesel fuel extender, namely its low vapour pressure and its low miscibility with water which will reduce the risk of phase separation. Furthermore, Butanol is completely miscible with diesel (Lenz and Moreira, 1980). Butanol is also more attractive than the lower alcohols due to its greater energy density. cf. Table 1 (Weast, 1982).

TABLE 1: HEATS OF COMBUSTION OF LOWER ALCOHOLS

	Hc(Kcal/mol)	K.cal/g
Methanol	173.64	5.43
Ethanol	326.68	7.10
n-Propanol	482.75	8.05
n-Butanol	639.53	8.64

The acetone/butanol/ethanol (ABE) mixture generated by the typical fermentation of carbohydrates by Clostridium acetobutylicum may also have advantages as a co-solvent for methanol/gasoline blends (Lawson, 1986).

It is not surprising, therefore, that interest has been regenerated into the production of butanol by the fermentation of carbohydrates derived from biomass materials. However, commercial technology for the production of butanol by fermentation has changed little during the past fifty years. Thus, it is still considered a fairly low yielding batchwise operation (Spivey, 1978). Product distribution (acetone, butanol and ethanol) and product yields reported in the literature vary with the feedstock, microbial species or strain, and operating parameters such as pH (Gottschal and Morris, 1982), temperature (Carnarius, 1940; McNeil and Kristiansen, 1985; Voget et al., 1985) and nutrients (Gottschal and Morris, 1981).

It is widely recognized that a major limitation with the acetone- butanol-ethanol (ABE) fermentation with bacteria such as Clostridium acetobutylicum, is the sensitivity of the organism's metabolic processes to inhibition by both intermediate and terminal metabolic products. Growth limitation of Cl. acetobutylicum was investigated by Costa (1981), and Costa and Moreira (1983). Their data indicated that a 50% reduction in cell growth occurred when the butanol concentration in the fermentation broth reaches 11 g/L. Acetone was not inhibitory at 29 g/L while ethanol did not reduce cell growth by 50% until a concentration of 51 g/L was attained. The intermediate products, acetate and butyrate, were the most toxic compounds investigated with concentrations of 5 and 8.5 g/L respectively causing cessation of growth.

It is generally accepted that combined solvent concentrations of 20 g/L are highly toxic to the micro-organisms. This obviously limits

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the yield of a batch process and requires higher costs to recover the product from large volumes of beers containing low concentrations of solvents. Furthermore, the low yielding process requires substantial capital investment in fermentation, distillation and by-product processing (e.g., evaporation) equipment (Lenz and Moreira, 1980).

A typical batch culture consists of two phases. There is an initial acidogenic stage in which acetate and butyrate accumulate in the broth accompanied by a rapid decrease in pH if the medium is weakly buffered. This is followed by the solventogenic stage whereby the organism converts the acetate and butyrate to acetone, butanol and ethanol and the pH of the medium will rise as the acids are consumed.

To overcome the significant economic limitations associated with batch ABE fermentations, scientists and engineers are directing their attention at several different approaches to improving the performance (yield) of the ABE fermentation. Approaches include the selection of microbial strains more tolerant to the solvents, improvement in the knowledge of the metabolic pathways controlling the production of solvents and new technologies to produce higher yields of solvent per unit of fermenter volume.

Several approaches have been investigated to increase the solvent production per unit of fermenter volume. In continuous culture, the goal is to maintain the solvent concentrations in the broth below a critical "toxic" level by selecting an appropriate rate of dilution of the broth with nutrient solution. Such methods seek to improve yields by maintaining the cells in the solventogenic phase, reducing the proportion of feedstock utilized for biomass generation, eliminating the "non-productive" acidogenic phase of a typical batch culture and eliminating the down time for cleaning, sterilization, etc. which is inherent with batch fermentations.

In recent years several research groups have demonstrated that Cl. acetobutylicum can be maintained under conditions of single or two stage continuous culture with solvent production maintained at levels close to those obtained in batch cultures (Bahl et al, 1982: Leung & Wang, 1981, Monot et al, 1983, Gottschal and Morris, 1982: Fick et al, 1985).

An alternative method to separate the "toxic" solvents from the microbial cells is the use of immobilized systems whereby the solventogenic bacteria are immobilized in support matrices such as calcium alginate beads (Haggstrom and Molin, 1980; Haggstrom, 1981). High conversion efficiencies have been observed with such columns although a decline in solvent production rate will normally occur with time, e.g., 1,000 hours. For such a concept to have practical application, problems such as the need for gas venting and the rate limiting diffusional barriers to nutrients and products moving in and out of the solid support must be addressed.

Taya et al (1985) have sought to overcome the inhibitory effects of the solvents by the use of an extractive fermentation system. Thus, when Cl. acetobutylicum is grown in the presence of immiscible, non-toxic solvents such as oleyl alcohol or guerbet alcohol, butanol, the more toxic solvent produced in the culture, partitions into the organic phase. These authors demonstrated in laboratory tests that the production of butanol from batch cultures of Cl. acetobutylicum, IAM 19012 could be increased by a factor of 2.6 when this extraction fermentation approach was used.

Whilst the above advances show potential improvements in productivity of solvents by ABE fermentations, they do not significantly improve the economic and logistics of solvent recovery. Large volumes of beer with low solvent concentrations (2 - 5%) must still be manipulated and the capital and operating costs will remain high. Similarly, the

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waste treatment costs for the large volumes of stripped beer will remain a significant economic factor.

This report describes investigations into the application of a novel fermenter-purifier to the ABE fermentation with the view to achieving the dual benefits of continuous fermentation coupled with simultaneous recovery and concentration of the volatile solvents.

4. THE ONTARIO RESEARCH FERMENTER-PURIFIER

Ontario Research has recently developed a new fermenter concept based on the airlift principle. The reactor is a cylindrical vessel fitted with a concentric open-ended draft tube (Figure 1) (Mulholland, 1984). Inert gas is vigorously sparged into the lower end of the draft tube. As the gas passes up the inner tube, the gas bubbles are coupled to the liquid by frictional forces and the low density due to voidage causes a cyclical flow pattern up the tube and down the annular space. This results in excellent mixing, heat and mass transfer and gas liquid contact (Margaritis and Sheppard, 1981). The device has been demonstrated to be an excellent and unique ethanol fermenter and is the subject of a pending patent application.

A very attractive feature of the design is the very long (infinite) residue time of the feedstock in the fermenter coupled with the very short residence time of the products. This is achieved by condensation of volatiles out of the stripping gas which is then recycled to the fermenter. The only requirement is that the product(s) of the fermentation should be volatile. The results are that the potentially toxic products are continuously removed from the fermentation broth while the condensate will consist of more concentrated, partially purified solutions of the products.

Previous results with ethanol have shown approximately a doubling of volatile uptake per 10^{o}C rise in operating temperature. It would be advantageous therefore to operate the system at as high a temperature as the micro-organisms can tolerate in order to increase the efficiency of stripping.

Heat dissipation from exothermic biochemical processes is countered by the heat demand for vaporisation and indeed additional low

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grade heat is required. This eliminates the need for expensive cooling systems required with standard batch fermenters and the resultant heat transfer problems. It is generally accepted that the minimum temperature for effective stripping is 40°C. The upper limit will depend on the thermotolerance of the organisms being cultured.

The fermenter-purifier has been demonstrated to permit the fermentation of glucose by Saccharomyces cerevisiae (ATCC 4126) at 40°C at which temperature under the continuous stripping mode of operation the productivity was identical to that of a batch culture at 25°C. In a standard quiescent culture at 40°C, inhibition of the fermentation due to ethanol toxicity would rapidly occur.

The application of the fermenter-purifier to the ABE fermentation, an obligative anaerobic fermentation, poses several questions for which information does not appear to exist in the literature. The ABE fermentation is a much more complicated metabolic process than the fermentation of glucose by S. cerevisiae. Strict precautions against access of oxygen to the culture must be taken and the risk of microbial contamination is greater in the case of the ABE fermentation which will be conducted within the pH range of 4.3 - 6.5.

A separate but economically relevant aspect to be considered is the efficiency of the condenser system. Tests with ethanol stripping indicated that the water cooled (6°C) condenser system was approximately 40-60% efficient (Mulholland, 1984). Butanol with its boiling point of 117°C should be efficiently recovered but the behaviour of acetone in the recycled gases was anticipated to create some potential problems. While the organism, Cl. acetobutylicum is said to tolerate relatively high acetone concentrations, the effect of such concentrations on the solvent ratio in the fermentation broth and the feedback reactions on the cells' metabolic cycles must be considered.

5. EXPERIMENTAL PROGRAM

5.1 Microbial Culture

Following a series of experimental tests (small scale), Clostridium acetobutylicum (ATCC 824) was selected for the program. It was demonstrated that this organism would ferment glucose at 40°C with the production of butanol, acetone and ethanol under the conditions of continuous gas stripping.

The original stock culture of this organism was kindly made available to us by Dr. C.W. Forsberg, University of Guelph.

5.2 Maintenance of the Microbial Culture

Cells were cultivated in reinforced Clostridial medium (RCM: Oxoid) at 37°C for 24-36 hr. Under anaerobic conditions, the bacterial suspension (9 mL) was then mixed with 80% (w/v) glycerol solution (1 mL). The cultures were stored frozen at -20°C. Starter cultures for individual experiments were prepared by inoculating the appropriate medium with a freshly thawed stock culture. The cells could be freeze-thawed about four times in the presence of glycerol.

5.3 Culture Medium

A defined medium was used for fermentation experiments (Forsberg, 1984).

Five solutions were prepared:

Solution A - K_2HPO_4 (7.63 g) and KH_2PO_4 (10 g) dissolved in distilled water and diluted to 1 L.

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Solution B - $(NH_4)_2SO_4$ (20 g), $MgSO_4.7H_2O$ (1.0 g), NaCl (0.1 g) $Na_2MoO_4.4H_2O$ (0.1 g), $CaCl_2.2H_2O$ (0.067 g) $MnSO_4.4H_2O$ (0.15 g) dissolved in distilled water and diluted to 1 L.

Solution C - Biotin (0.005 g), Thiamine hydrochloride (0.1 g), and p-Aminobenzoic Acid (PABA) (0.1 g) dissolved in distilled water and diluted to 1 L.

Solution D - Glucose (250 g) dissolved in distilled water and made up to 1 L.

Solution E - $FeSO_4.7H_2O$ (0.6 g) dissolved in distilled water and diluted to 100 mL.

The pH of Solutions A-C were adjusted to 6.86 prior to sterilization. Solutions A,B, and D were sterilized by autoclaving at 18 p.s.i. for 15 minutes. Solutions C and E were sterilized by filtration.

To prepare 1 L of defined medium, Solution A (100 mL), Solution B (100 mL), Solution C (10 mL) and Solution D (200 mL) were mixed asceptically and diluted with sterile distilled water to 1 L. Solution E (1 mL), prepared and sterilized immediately prior to being required was added to the bulk solution. Thus the final composition of the medium was:

	g/L		g/L
K_2HPO_4	- 0.763 g	$CaCl_2.2H_2O$	- 0.0067 g
KH_2PO_4	- 0.100 g	$M_2SO_4.4H_2O$	- 0.015 g
$(NH_4)_2SO_4$	- 2.0 g	Biotin	- 0.00005 g
$MgSO_4.7H_2O$	- 0.1 g	Thiamine HCl	- 0.001 g
NaCl	- 0.01 g	PABA	- 0.001 g
$Na_2MoO_4\ 2H_2O$	- 0.01 g	$FeSO_4.7H_2O$	- 0.006 g
		Glucose	- 50 g

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5.4 Analytical Procedures

a) Sampling

Bacterial suspensions recovered from cultures or fermentations were filtered to remove the bacterial cells (Millex 0.22 μm pore size filters). In some instances, the fermentation broths became very viscous and in those situations, the samples were initially centrifuged at 15000 rpm for 20 minutes. The supernatant solutions which were still slightly viscous, were then filtered through a 0.45 μm pore size filter (Millipore) with a glass micro fibre filter (Whatman) as prefilter.

b) Glucose and Alcohols

Fermentation broths and condensates were analyzed for their glucose, butanol, acetone and ethanol concentrations by high pressure liquid chromatography (HPLC) using a Waters Model 590 system equipped with a WISP 710B Automatic Sampler and a Diffractional Refractometer Detector, Model 401. Quantitation was by intergration. The column was an Aminex® Ion Exclusion HPX 87H, 300 x 7.8 mm (BioRad) and the mobile phase was either 0.008 M phosphoric acid or 0.012 N sulphuric acid at ambient temperature. For confirmation purposes, the fermentation broths in the later experiments were also analyzed by gas liquid chromatography to determine the butanol, acetone and ethanol concentrations. These analyses were performed on a Varian Model 3700 Gas Chromatograph equipped with a flame ionization detector. The column was Carbopack C coated with 0.3% Carbowax 20M/0.1% H_3PO_4. During an analysis, the column temperature was raised from the starting temperature of 40°C to a final temperature of 175°C at the rate of 10°C/min.

c) Organic Acids

Organic acids in the fermentation broth were determined by gas liquid chromatography using the same column and conditions as described for the alcohols.

d) Ammonium and Phosphorus

Ammonium and phosphate (as orthophosphate) concentrations in the filtrates of the fermentation broths were determined according to Methods SM 417 and SM 424E respectively, in Standard Methods for the Examination of Water and Wastewater, 16th Edition, 1986. The procedure for ammonium analysis involved preliminary distillation and entrapment of the liberated ammonia in boric acid followed by titrimetric determination of the ammonia concentration.

The assay for inorganic orthophosphate was performed directly on the filtrate of a broth using the stannous chloride method (Method SM 424E).

e) ATP Assay for Viable Biomass

The adenosine triphosphate (ATP) assay procedure for the estimation of viable biomass was used as required to monitor the metabolic status of a fermentation (Cairns, 1985). The sampling procedure involved transferring an aliquot (0.2 mL) of a bacterial suspension into 95% (v/v) ethanol (10 mL) immediately after the sample had been withdrawn from the fermenter. The ethanol suspension was shaken well to release the ATP from the bacterial cells. This procedure quenched the metabolic activity of the cells and stabilized the ATP for assay by the firefly luciferase enzymatic procedure. To do so, the ethanolic solution was diluted appropriately into 0.005M sodium triphosphate buffer pH 11.7 which was 0.005M with respect to magnesium ethylene diamine tetraacetic acid, disodium salt (Mg EDTA). A second higher dilution was also made with the phosphate/Mg EDTA buffer solution. Both dilutions were then assayed for ATP by the firefly luciferase method using a Turner Designs, Model 20 photometer. The reaction was initiated by injecting 50 µL of luciferin/luciferase

reagent (Turner Designs California) into 50 µL of the diluted ATP extract in a cuvette. If the measured ATP contents of the duplicate dilutions were not proportional to their respective dilution factors, further dilutions in the phosphate/Mg EDTA buffer were made and analyzed until proportionality was observed between the different dilutions. The ATP contents were determined by comparing against standard concentrations of ATP in the same buffer solution.

5.5 Preliminary Fermentation Studies

Tests were performed with standard laboratory apparatus to assess the effect of sparging on the growth of Cl. acetobutylicum and the subsequent production of solvents. Initial cultures were sparged with carbon dioxide but subsequently nitrogen was used. Cultures were incubated at 39-40°C and the exhaust gases were passed through a condenser (cooled with tap water) to collect the volatile components.

Subsequently tests were performed whereby the exhaust gases were recycled (100-150 mL/min.) by a small air pump through a fermentation. The return gases were sterilized by filtration through sterile cotton.

5.6 Design of Small-Scale Fermenter-Purifier

Previous tests with an ethanol fermentation had utilized a fermenter-purifier whose operating volume was 11 L (Figure 1). However, it was expected that the operating mode of the fermenter-purifier system would influence significantly the metabolic pattern of an organism such as Cl. acetobutylicum. Accordingly, before proceeding to fermentations of this scale, a scaled-down fermenter-purifier was designed whose dimensions (height, diameter, etc.) would match closely those of the 11 L fermenter system. The diagrammatic for this small-scale prototype fermenter is shown in Figure 2.

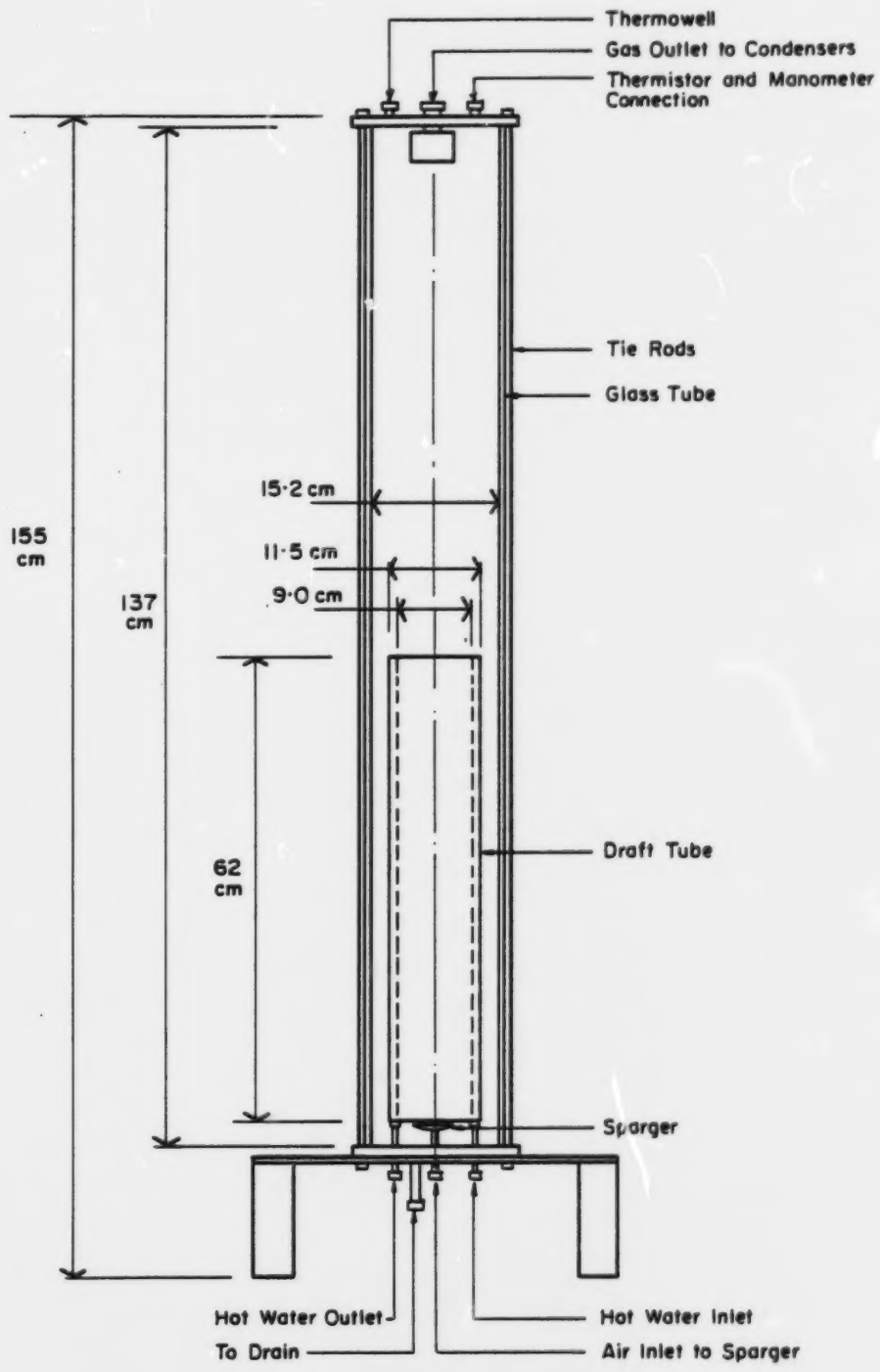

FIGURE 1. EXPERIMENTAL APPARATUS FOR LOW COST RECOVERY OF ETHANOL.

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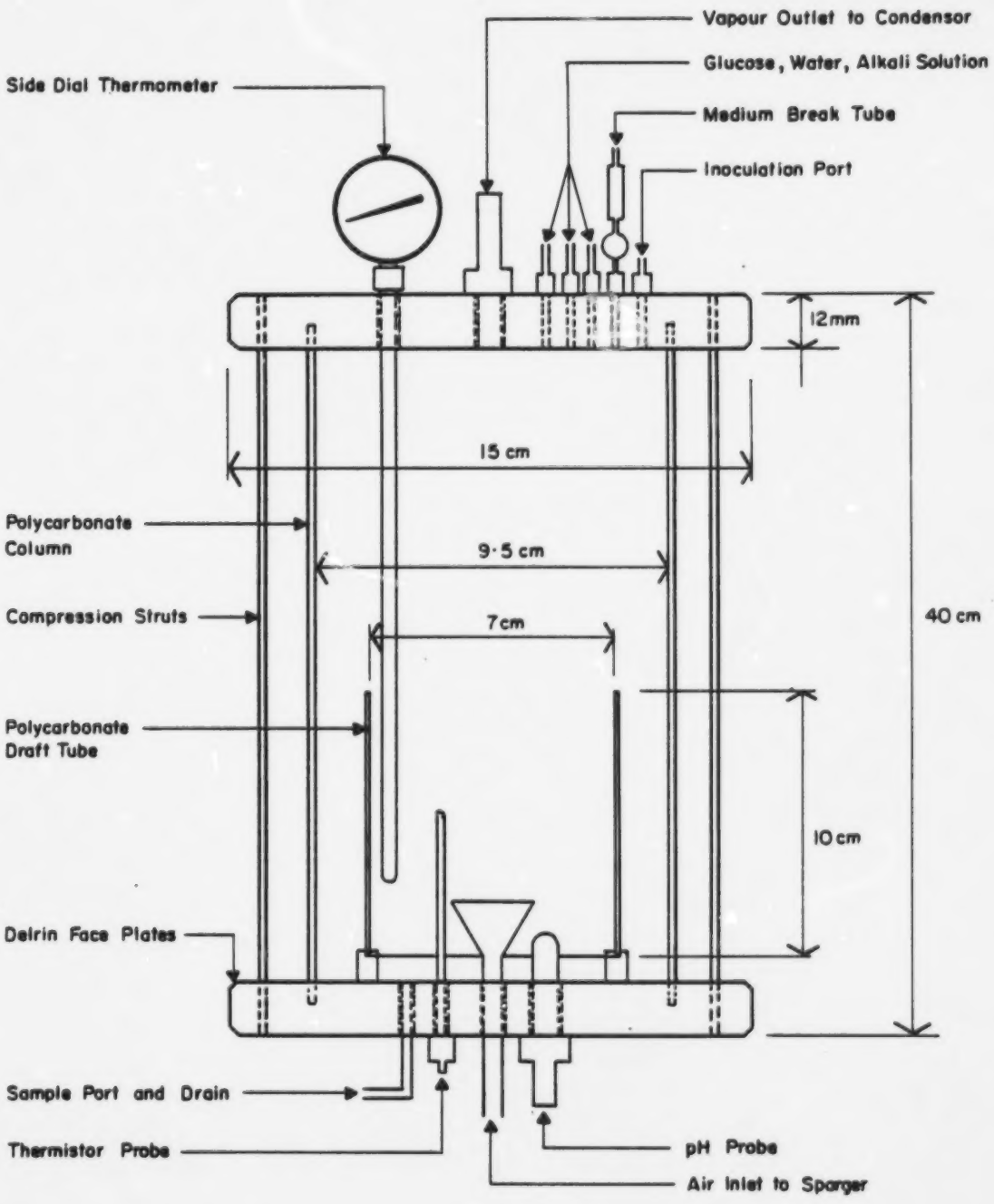

FIGURE 2. BENCH SCALE FERMENTER-PURIFIER (MAX. CAPACITY : 2ℓ)

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An initial model was constructed from relatively inexpensive synthetic materials. The head and base plates were cut from Delrin® and machined to accept the various inlets, outlets, sensors, etc. The walls of the fermenter were cut from polycarbonate tubing and carefully machined so that a tight seal was formed between the tubing surface and O rings in the head and base plates when tension was applied. The draft tube was made of plexiglass tubing and the supports to mount and centre the draft were cut from Delrin® rod. All fittings were, with the exception of the fritted glass sparger, made of polyolefins.

In a preliminary test with Saccharomyces cerevisiae, a very active fermentation resulted. However, when several attempts to establish actively fermenting cultures of Cl. acetobutylicum had failed, the materials of construction were investigated for possible inhibition of the growth of this latter organism. The indication that the plexiglass components were inhibitory to the growth of Cl. acetobutylicum to varying degrees led to modifications being carried out. Coating the surfaces in contact with culture medium with Teflon® spray failed to solve the problem and eventually a new apparatus was constructed from 316 stainless steel head and base plates with the side walls and draft tube composed of borosilicate glass. The dimensions were modified slightly to reflect the differences in dimensions of the similar components constructed of glass or synthetic. The glass draft tube was initially held in place with stainless steel guides, but after these guides darkened during the first fermentation, it was concluded that they were not resistant to components or products in the fermenter broth. Accordingly, 316 stainless steel struts and side supports were machined and glued in place with epoxy resin.

For the first set of tests the polyolefin inlets, fittings, etc. were left in place but when it became desirable to be able to sterilize the entire fermenter by autoclaving, stainless steel (316) fittings were installed in the head and base plates.

The pH probe for this design is inserted in the base plate, i.e., is inverted. Some difficulty was encountered in obtaining a gel electrode functioning in the desired manner. An electrode was custom made. However, this electrode was damaged during one of the earlier fermentations and an alternative source of supply was found to be the electrode designed for base insertion in a commercial airlift fermenter (Bethesda Research Laboratory).

Temperature was maintained on the unit with heating pads under microprocessor control. Gas recycle was achieved with a Model 107 CA 11, 1/20 HP diaphragm vacuum/pressure pump (Thomas Industries Inc.), with the flow rate controlled by a needle valve inserted in the gas line.

5.7 Additional Fermenter Capacity

While the various modifications were being carried out on the fermenter-purifier prototype, a new design of a small scale (1.8 L operating volume) airlift fermenter became available commercially from Bethesda Research Laboratories (BRL). This unit comes equipped with heater control systems, air compressor pump, foam and level controller and pH controller (cf. Appendix II). One of these units was purchased for the project. The major differences in the fermenter vessel compared to the ORF fermenter-purifier design were the use of an off-center baffle plate instead of the central draft tube in the fermenter- purifier, and dimensions of the fermentation chamber being approximately 54.6 x 7.9 cm compared with 37.6 x 9.5 cm in the case of the fermenter-purifier prototype. The baffle plate was offset such that the riser portion of the split cylinder occupied approximately 67%, while the downcomer represented approximately 33% of the total volume. The minimal operating volume for the BRL fermenter was 1.8 L compared with 1.0 L in the case of the fermenter- purifier. This meant that the headspace was significantly less in the case of the BRL fermenter. A BRL unit was obtained on trial and found to function well for a S. cerevisiae fermentation with recycle.

5.8 Condenser Systems

An integral part of the fermenter-purifier concept is the availability of condenser systems able to efficiently remove the entrained volatile components from the recycle gas stream. The acetone-butanol fermentation introduces the added dimension of one product, i.e., acetone, which has a very high vapour pressure but very low dew point.

In an initial fermentation operated with continuous gas recycle at 40°C, butanol production was lower than expected but more significantly the acetone concentration in the fermentation reached relatively high concentrations. This was attributed to the relatively inefficient condensation of acetone from the gas phase by the laboratory size water cooled condenser in use.

5.8.1 "Conventional" Condenser Systems

With a view to being able to control the rate and efficiency of removal of the solvents from the gas phase, a series of experiments were initiated to assess the ability of different "condenser" designs to remove acetone from the recycle gases. The fermenter-purifier was used in this series of tests. An initial group of tests were conducted at flow rates of approximately 19 L/min (16 VVM) but subsequently all tests were conducted at a flow rate of 1.6 VVM equivalent to the typical flow rate used in fermentation trials. Several different condenser "configurations" were inserted in the flow path. The condensers included standard double walled condensers cooled with ice water or alternatively cooled to -7°C with recycled coolant from a refrigerated cooling system; 500 mL Kjeldhal flasks packed with glass beads or Raschig rings to create a larger surface area and cooled in acetone/dry ice mixture; a cold thimble cooled with liquid nitrogen; Graham condensers cooled with

methanol which was cooled in turn by passage through a copper heat transfer coil immersed in acetone/ dry ice. The schematics of the test circuits are shown in Figures 3 and 4.

The 2,4 dinitrophenylhydrazine (2,4-DNPH) solution was placed in the gas stream immediately after the condensers. In the presence of acetone, the 2,4-DNPH forms a heavy precipitate. In our initial tests, the time taken for this precipitate to develop was used to gauge the efficiency of a particular condenser combination.

Initial tests were performed with high acetone concentrations, e.g., 100 g/L to challenge the system. Subsequent tests were performed with original acetone concentrations down to 1 g/L (Figure 3) and final tests were performed with butanol/acetone/ethanol mixtures in the typical ABE ratio of 6 g: 3 g: 1 g/L respectively (Figure 4).

In all tests the fermenter + solvent solution were maintained at 40^{o}C and the length of time that the gas recycle was operated varied between experiments although it was generally between 1 - 5 hours. At the end of the selected time period, each condensate was collected and analysed where possible, for appropriate solvent concentrations by HPLC. The residual solution in the fermenter vessel was also analyzed.

Based on the data from these tests it was concluded that it was desirable that the condenser system be operated at as low a temperature as can reasonably be attained with laboratory systems. Two high efficiency, double- walled condensers were constructed to order from borosilicate glass. Each condenser consisted of an inner thimble around which was wrapped an additional cooling coil, and an outer jacket. The dimensions of the outer jacket were 7.5 cm OD x 61 cm long. The total cooled surface in contact with the gas flow was estimated to be 1650 cm^{2} and the distance between the outer edge of the coil and the inner

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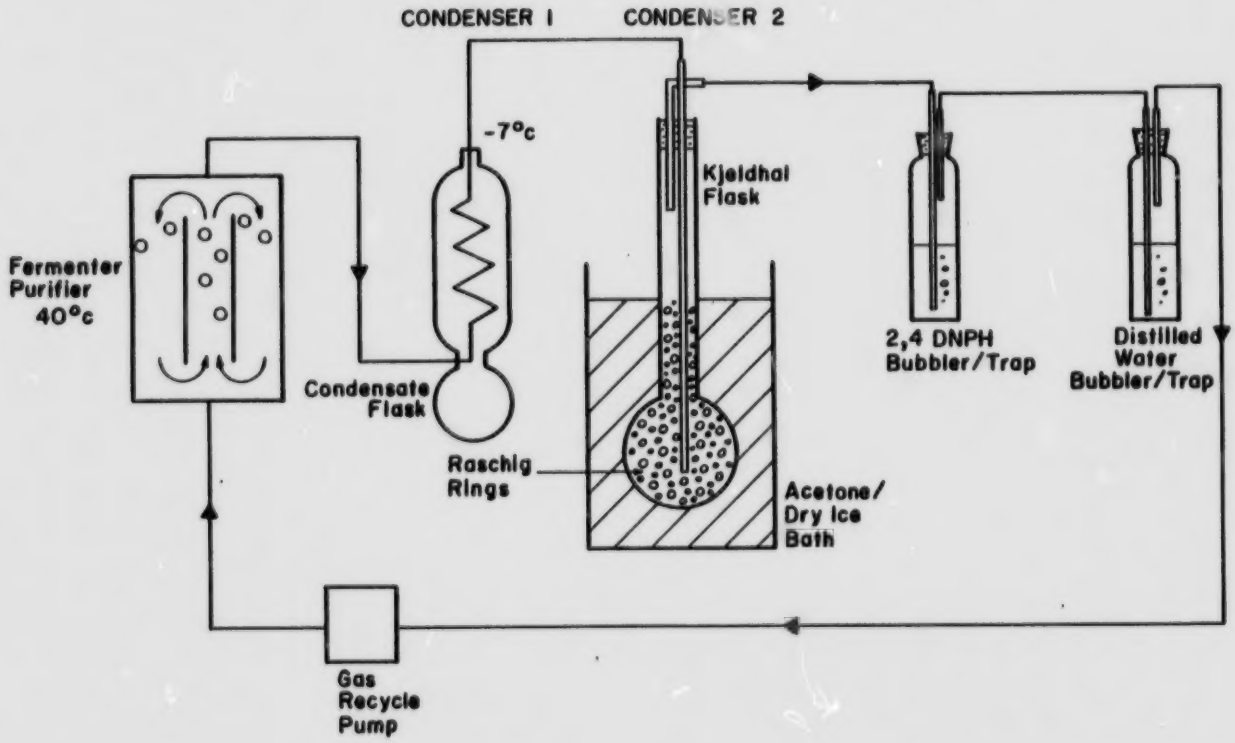

FIGURE 3. REPRESENTATIVE SCHEMATIC OF INITIAL CIRCUIT FOR EVALUATION OF ACETONE RECOVERY FROM GAS RECYCLE STREAM.

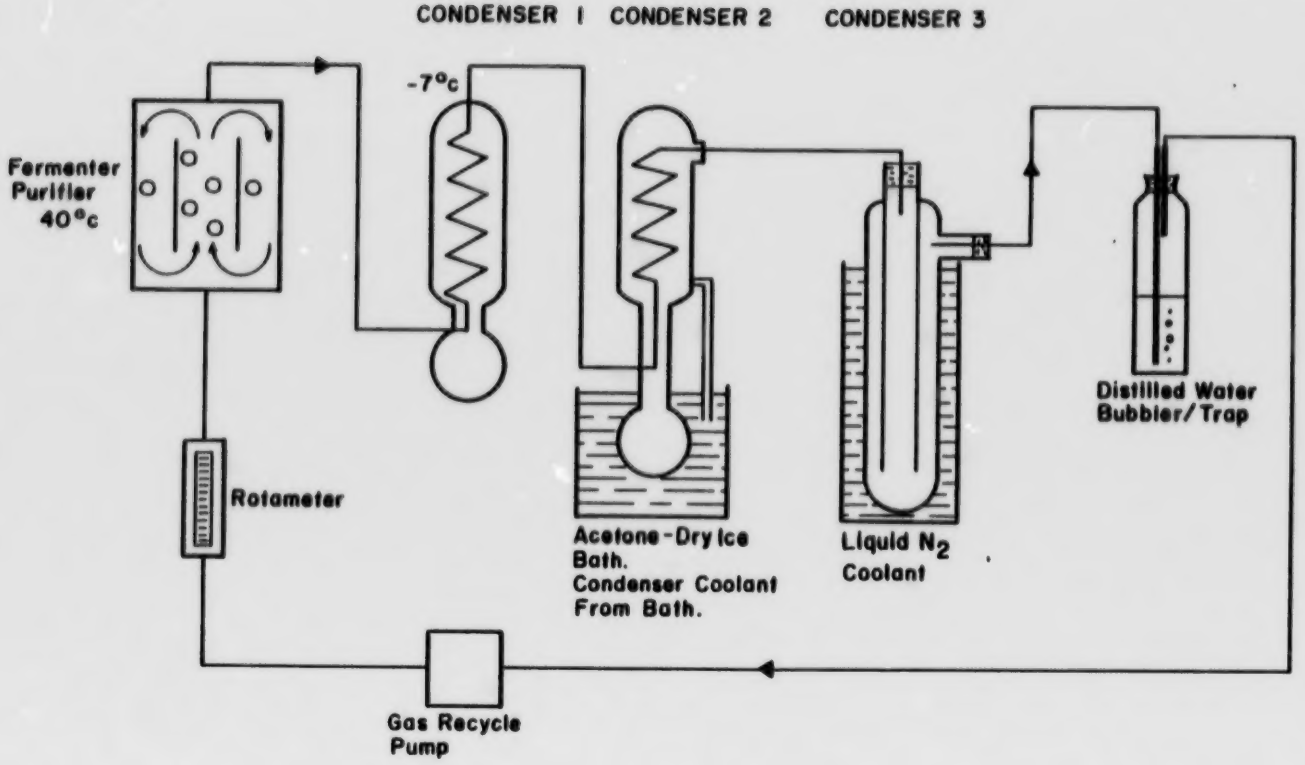

FIGURE 4. REPRESENTATIVE SCHEMATIC OF FINAL CIRCUIT FOR EVALUATION OF SOLVENT RECOVERY FROM GAS RECYCLE STREAM.

surface of the outer jacket was only 2 mm. The gas hold up volume within the condenser and in contact with the cooling surfaces was estimated to be 632 mL, thus, at a flow rate of 1.6 L/min the mean residence time of the recycled gas in the condenser would be approximately 40 seconds.

Two jacketed receiving flasks were also constructed to order such that condensates would be maintained at lower temperatures to minimize re-entrainment of the more volatile components.

5.8.2 "Novel" Condenser Systems

The need for relatively low coolant temperatures to achieve satisfactory condensation was viewed as undesirable in the long term for an economically viable process. Therefore, alternative condensation systems were also investigated (particularly for the removal of acetone from the vapour phase). One approach with significant potential was the use of hydrophobic molecular sieves.

Tests were performed on the ability of silicalite SR-115 hydrophobic molecular sieve, supplied by Union Carbide Ltd., to adsorb acetone, butanol and ethanol from the vapour phase. The system was operated as for the condenser tests except that a gas washing bottle served as the reservoir (Figure 5). The aqueous solution of a solvent was maintained constant at 40-45^{o}C. The exhaust vapour stream was passed through a column of silicalite pellets (421.3 g, 5.5 x 27 cm) and then recycled to the gas washing bottle. The solvent uptake capacity was assessed and adsorbed solvent was recovered from off the silicalite by heating the column with external heating pads (set at 150 - 220^{o}C) and a flow of nitrogen through the column. Two condensers were used to recover desorbed volatile components from the nitrogen stream (Figure 6). The first consisted of a Graham condenser cooled with tap water and the second a cold thimble immersed in liquid nitrogen. The volumes of

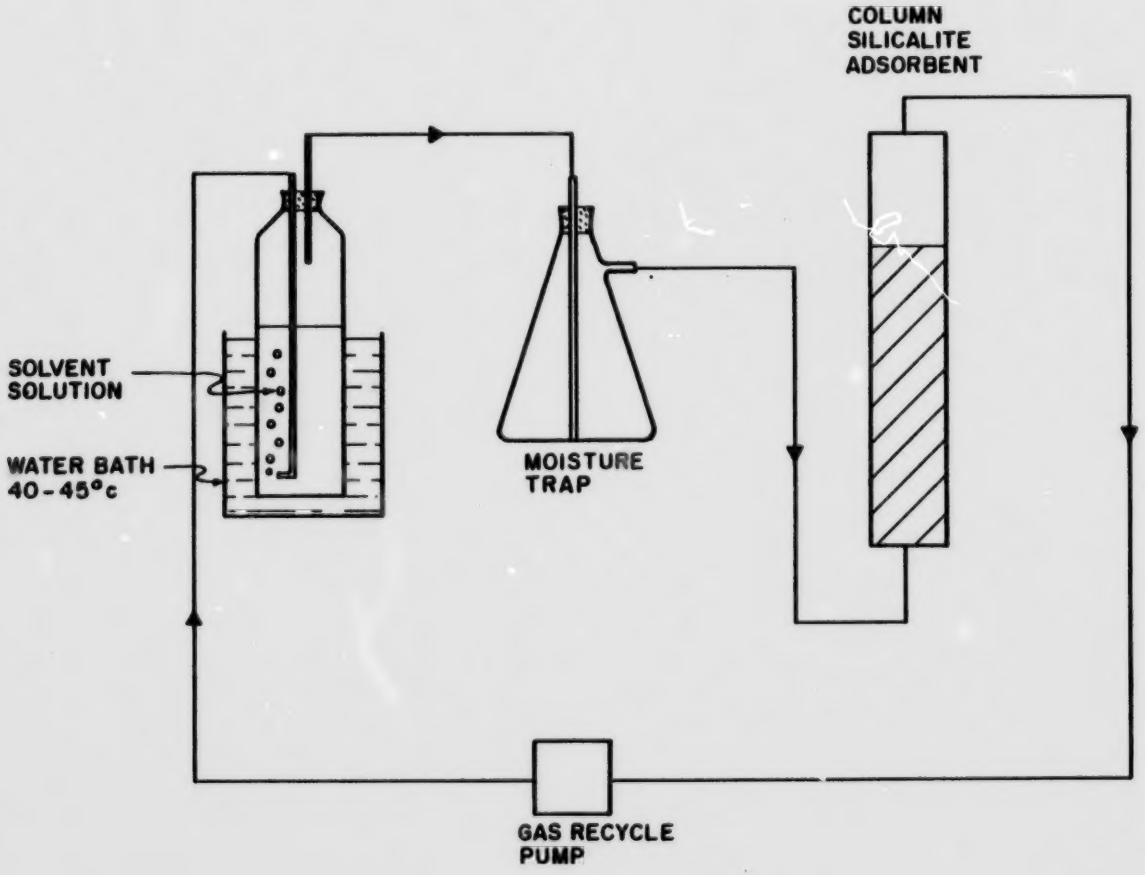

FIGURE 5. SCHEMATIC OF CIRCUIT TO EVALUATE SOLVENT UPTAKE BY SILICALITE.

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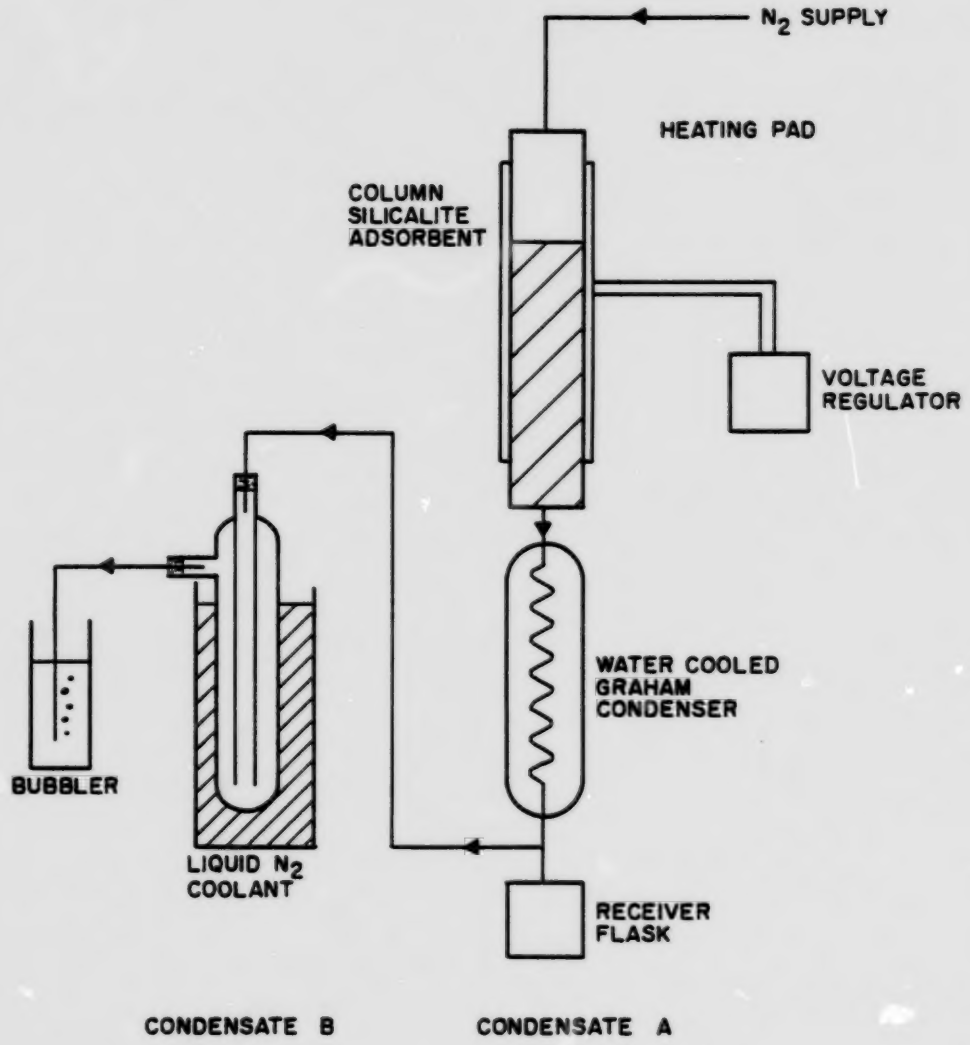

FIGURE 6. SCHEMATIC OF CIRCUIT FOR SOLVENT RECOVERY FROM SILICALITE BY THERMAL DESORPTION.

condensates were measured and the solvent concentrations analysed by HPLC to identify the concentration factor achieved along with the efficiency of solvent recovery.

5.9 Fermentation

Based on the analytical data obtained from the initial small scale fermentations, the solvent condensation experiments and the silicalite adsorption tests, two process schematics were designed and assembled.

The first, the fermenter-purifier circuit (Code Letters PF) is shown in Figure 7. The two glass-walled condensers, described in the previous section were placed in series after the fermenter-purifier. The goal was to be able to cool these columns to the lowest possible values that could be achieved by circulating chilled coolant from refrigerated reservoirs. Accordingly, the first condenser was operated typically with coolant entering it at $-7^{o}C$. The temperature of the coolant returned to the reservoir was generally $4^{o}C$. The second column was maintained at a temperature of $-16^{o}C$ in the reservoir. To achieve this temperature, it was necessary to insulate all feed lines between the condenser, the receiving flask and the chilled reservoir, with tubular polystyrene foam. Similarly, the condenser column and receiving flask were both wrapped with fiberglass pipe wrap insulation.

Gas flow was maintained by a diaphragm compressor/vacuum pump (Thomas Industries Inc., Model 107 CA11 1/20 HP). The direction of gas flow had the exhaust gas from the fermenter-purifier entering at the base of the first condenser and exiting from the top of the condenser to enter at the base of the second condenser. In this way, it was intended to minimize the risk of re-entrainment of volatile components in the condensates since the condensed liquids would drain into the cooled reservoirs and be removed from direct contact with the main gas flow.

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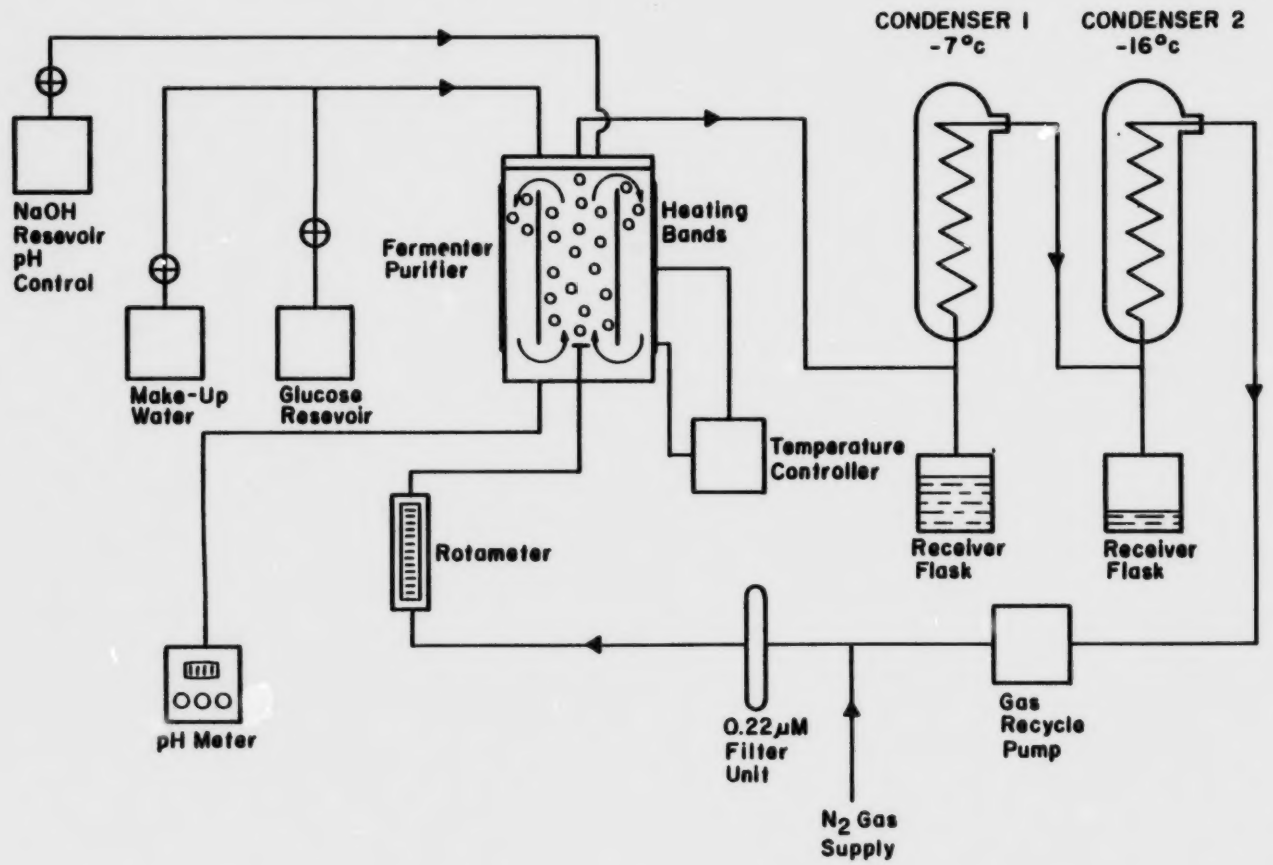

FIGURE 7. SCHEMATIC OF ORF FERMENTER PURIFIER CIRCUIT.

A flow meter was inserted in the circuit to monitor the gas flow which was controlled by a needle valve situated before the pump. The system used materials of construction which would allow the fermenter-purifier, the inlet gas line between the fermenter and the pump, all feed lines, reservoirs of makeup water, glucose solution, and alkali to be sterilized by autoclaving. The recycled gas stream and any nitrogen gas bled into the system were sterilized prior to introduction into the fermenter-purifier by passage through a 0.22 μm pore size, 25 mm diameter Acro Disc® filters (Gelman) placed in the return gas line immediately after the pump.

A nitrogen line was inserted between the pump and flowmeter to allow sterile, high purity nitrogen gas (purified of oxygen, by passage through a Matheson, Model 6406 gas purifier) to be fed into the system to keep fermentations anaerobic and to purge the total recycle system of oxygen. All lines in the circuit were composed of either polypropylene or butyl rubber to minimize any risks of oxygen permeation into the system. Nitrogen flow was switched on to create a positive pressure during the sampling of fermenter broth and condensates, the latter being collected via stopcocks at the base of each reservoir.

All joints in the fermenter-purifier circuit were tested to ensure freedom from leaks. This included the inclusion of a pressure gauge between the fermenter and the first condenser and enabled a measurable positive pressure to be placed on the system prior to sampling the fermenter broth or the condensates.

The second schematic (code named AF) utilized the BRL Airlift fermenter (Figure 8). Three "condensers" were placed in series. The first condenser, an air condenser, was intended as the overflow trap but it was found during normal operation (when the tendency for excessive foaming had diminished) that some moisture + solvent tended to condense

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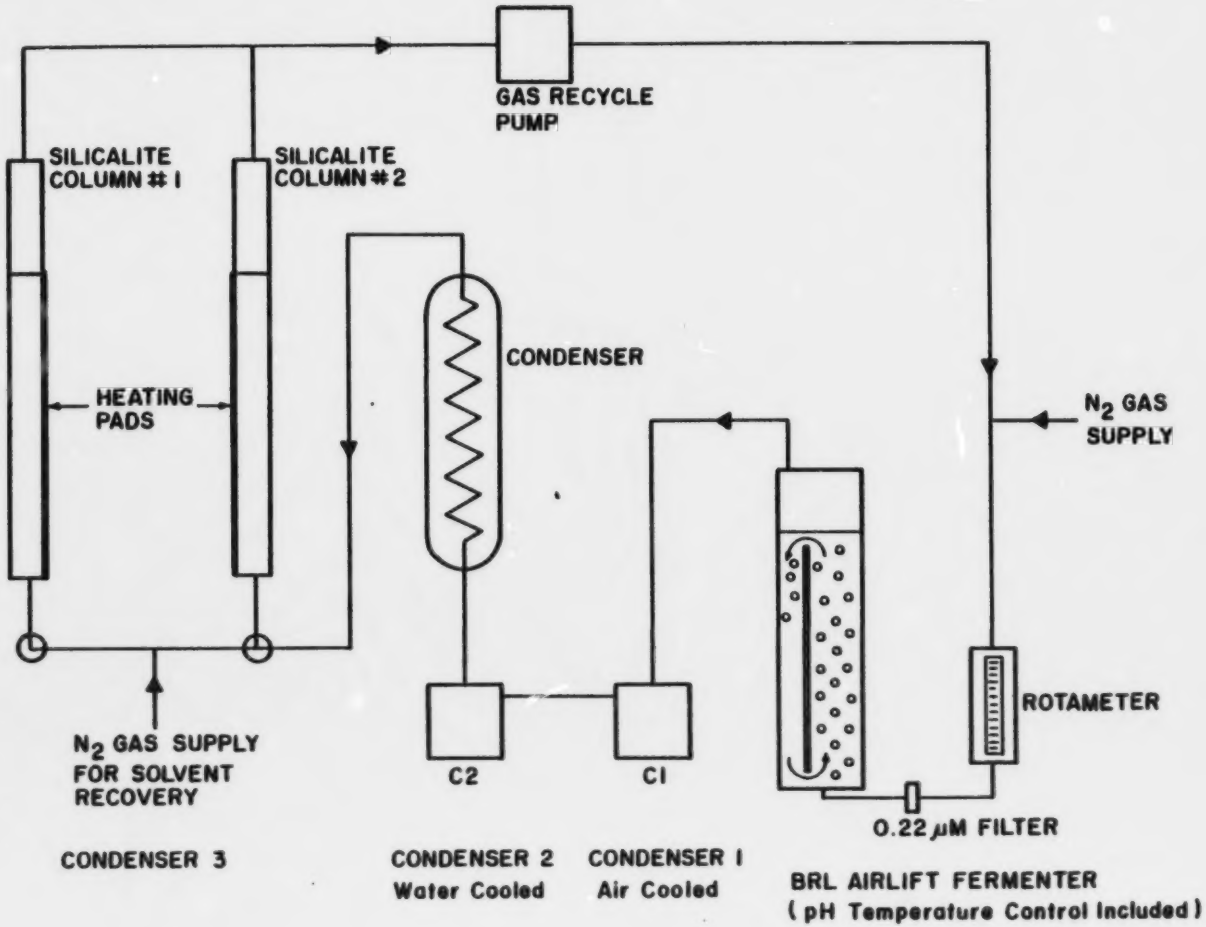

FIGURE 8. SCHEMATIC OF AIRLIFT FERMENTER PURIFIER CIRCUIT .

out in this flask. The gas flow then entered the second, water-cooled, double-walled condenser at the base of the column. Condensate collected in the receiver flask while the gas flow moved from the top of condenser 2 to the base of condenser 3. Condenser 3 consisted of dual columns each containing 153 g of silicalite SR-115 pellets. The dimensions of each bed of pellets were 2.5 x 30 cm. The connections were such that the gas stream could be passed through one column and at an appropriate interval the flow would be switched to the second column. In the meantime, the "saturated" column could then be stripped using the combination of external heating by heating pads around the column coupled with a nitrogen gas flow to displace the volatilised solvents. (As before, a water-cooled condenser and a liquid nitrogen-cooled condenser were used in series in order to recover desorbed solvents).

Stripped gases were then recycled via an external diaphragm compressor/vacuum pump (Thomas Industries Inc., Model 107 CA11, 1/20 HP) to the gas inlet of the airlift fermenter. Between the pump and the gas inlet connection, a nitrogen connection allowed high purity nitrogen (scavenged for oxygen by passing gas over heated copper wire) to be bled into the fermenter to maintain a both positive pressure and anaerobic condition during sampling, etc.

For the initial tests (PF0, PF1 and AF1) the addition of makeup water, alkali, acid, antifoam solution, glucose feed and any other nutrients were by manual addition. In tests PF2 and AF2 the control was still manual but the system in PF2 allowed for the addition of glucose feed, alkali by peristaltic pumps .

All fermentations were conducted at 40^{o}C. Fermenters and relevant fittings, tubing, etc. were sterilized by autoclaving. The BRL pH electrode, was stated to be unable to withstand repeated sterilization, hence it was soaked in 75% (w/v) ethanol solution for

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several hours prior to insertion aseptically into the base plate of the sterilized fermenter vessels. Each fermenter was then charged with the appropriate volume of sterile medium. Cultures were then inoculated with a 10% (w/v) inoculum of an actively growing culture of Cl. acetobutylicum in the same medium (PF1, AF1) or RCM medium (PF2 and AF2).

Fermentations were monitored daily for solvents and glucose concentrations by HPLC, pH, etc. and the glucose concentration was maintained at approximately 50 g/L. Data on organic acids and solvents by gas chromatographic analysis of the same samples were obtained at a later date.

6. RESULTS AND DISCUSSION

6.1 The Microbial Culture

Cl. acetobutylicum (ATCC 824) was selected for this study when it was demonstrated in small scale experiments that the organism would ferment glucose to butanol, acetone and ethanol at 40°C under the typical gas stripping conditions of the fermenter-purifier.

The concept of culturing an organism at a temperature greater than the accepted optimum for growth (37°C) when coupled with the continuous removal of volatile products may be expected to disturb the metabolic control mechanisms within the cells. In view of the considerable investigations which have been carried out on the metabolic control mechanisms of this particular strain, it was appropriate at the outset to use the established organism. Furthermore, it has been demonstrated (Monot and Engasser, 1983; Leung and Wang, 1981) that this organism could under conditions of continuous cultivation produce butanol and acetone concentrations similar to those obtained in batch cultures.

6.2 Analytical Procedures

At the outset of this program, high performance liquid chromotography had been selected as the procedure to monitor fermentation broths and condensates as required for the concentrations of glucose, acetone, ethanol and butanol. The use of solely this technique was continued up to the carrying out of fermentations PF1 and PF2, and AF1 and AF2. Prior to those tests, it was identified that certain organic acids liable to occur in the fermentation broths at different stages of a culture, would interfere with the quantitation of acetone and ethanol by HPLC. Thus, on the anion exclusion column HPX 87H and with the stated eluants, isobutyric acid could not be distinguished from ethanol.

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Butyric acid and acetone were also eluted at similar retention times such that the method could not separate them. No interference was noted from the butanol peak with any of the organic acids investigated, i.e., acetic, butyric, iso-butyric and valeric.

Although the concentrations of organic acids are typically at low levels in a batch culture at the phase of solvent production, at certain stages of the fermentation the concentrations of isobutyric and butyric acids will be high enough to bias positively the observed ethanol and acetone concentrations. Accordingly, all data for solvents and acids in fermentation broths were obtained by gas chromatographic analyses.

Broths can be pre-treated with an anion exchange resin to separate the interfering acids from the solvents but for future studies, it is recommended that gas chromatography be used for the quantitation of solvents in fermentation broths.

These reservations would not apply to the analysis of condensates where the purification achieved due to the lower volatility of the organic acids would provide solutions essentially free of interfering compounds.

6.3 Condensation

The fermenter-purifier has been demonstrated to permit the continuous fermentation of glucose to ethanol by Saccharomyces cerevisiae (ATCC 4126) at 40^{o}C. At this temperature in batch culture the organism is rapidly inhibited by the toxic action of the ethanol produced (Mulholland, 1984). This is achieved by the removal of volatiles in the stripping gas and the subsequent condensation of the volatiles compounds from the stripping gas prior to its recycle to the fermenter. To prevent accumulation of toxic compounds in the fermentation broth, it is essential that the rate of stripping/condensation of the ethanol exceeds the rate of ethanol production.

Tests performed with the vapour phase stripping of 2 - 8 wt% ethanol solutions at 40°C indicated that the condensation efficiency of a water cooled (6°C) condenser was generally 40-60% (Mulholland, 1984). This operating temperature was selected to simulate as closely as possible, condenser operating parameters in a commercial process.

The acetone butanol fermentation introduces the addition variables of the respective condensation efficiencies of acetone and butanol. The respective vapour pressures of the three solvents at 40°C are:

Ethanol - 135.3 mm Hg
Butanol - 18.6 mm Hg
Acetone - 410.0 mm Hg

Assuming a typical butanol/acetone/ethanol ratio of 6:3:1 and a maximum tolerable solvent concentration in a fermentation broth of 2% (w/v), total solvents, the partial pressures relative to their mol.fraction would be:

Ethanol - 0.0357
Butanol - 0.0183
Acetone - 0.2573

While recognizing the need to take into account how the individual components will behave in a fermentation broth, it is obvious that the acetone will be the most volatile component. Concern was felt that a standard water condenser might not be able to adequately condense out the acetone. On the other hand, although the butanol is less volatile, it may be expected that this higher boiling point (117°C) component will condense out more efficiently from the recycling vapour phase than does ethanol.

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The initial fermentation PFO (Figure 9), while showing positive indication of solvent production, provided data to support the hypothesis that the acetone would not condense out readily. Thus the acetone was seen to accumulate in the fermentation broth to levels as high as 6 - 8 g/L. Accordingly, a series of experiments were conducted to assess the efficiency of various condenser configurations and combinations in order to achieve more efficient recovery of acetone from the vapour phase.

6.3.1 Tests of Condenser Efficiencies With Respect to Acetone Removal from the Vapour Phase

A schematic of the circuit used to evaluate the removal of acetone from a recycling vapour stream is shown in Figure 3. Tests were conducted with the acetone solution in the fermenter-purifier maintained at 40^{o}C throughout the stripping period.

In all tests, the condensers were placed in series in order of decreasing coolant temperature. The experiments assessed the effects of coolant temperature and condenser surface area upon the removal of acetone.

The range of tests that were carried out with acetone solutions are shown in Table 2. Initial trials were performed with 10% (w/v) acetone solutions in order to generate sufficient volumes of condensates within a shorter stripping time, e.g., 1 - 2 hours. The flow rate was set arbitrarily and when a flow meter was included in the circuit, the gas flow rate was found to be 19.2 L/min, i.e., 16 VVM. While this increased the rate of stripping as indicated by the weights of condensates in Tests 1-7 (Table 3) the rate was excessive and therefore it was not unexpected that a precipitate appears in the DNPH bubble within a short period, i.e., 5-10 min. Reduction of the flow rate to 1.6 L/min (= 1.58 VVM) reduced significantly the yields of condensates with even 10% (w/v) acetone solution (Tests 7-9).

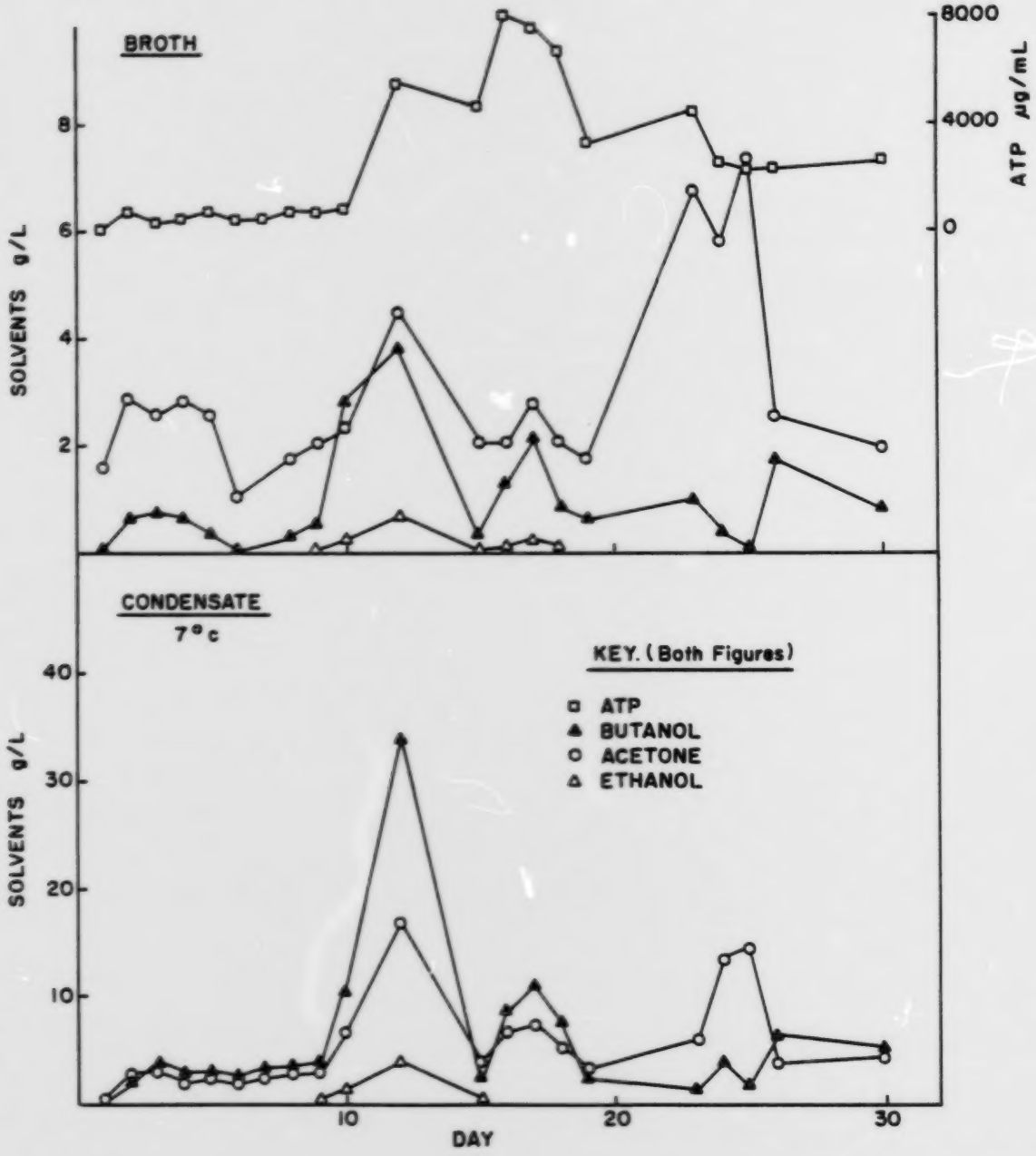

FIGURE 9. FERMENTATION PFO – FERMENTER-PURIFIER. ANALYSES OF BROTH.

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TABLE 2: STRIPPING TESTS - ACETONE-DESCRIPTION OF CONDENSERS

TEST	INITIAL ACETONE CONCENTRATION (% w/v)	STRIPPING TIME (min.)	FLOW RATE. L/min.	CONDENSER 1 DESCRIPTION	CONDENSER 1 TEMP °C	CONDENSER 2 DESCRIPTION	CONDENSER 2 TEMP °C	CONDENSER 3 DESCRIPTION	CONDENSER 3 TEMP °C
1	10	60	19.2	Large coil condenser (Buchi Rotavapor)	2	500 mL Kjeldhal flask. Coolant - Dry ice/acetone	-65		
2	10	60	19.2	As above	2	500 mL Kjeldhal filled with Raschig Rings Coolant - Dry Ice Acetone	-65		
3	10	60	19.2	Double walled Inner coil glass condenser, coolant 25% ethanol	-7	As above	-65		
4	1	60	19.2	As above	-7	As above	-65		
5	2.5	60	19.2	As above	-7	As above	-65		
6	5	60	19.2	As above	-7	As above	-65		
7	10	60	19.2	As above	-7	Double walled Inner coil glass condenser, coolant - 25% ethanol	-7	500 mL Kjeldhal flask filled with Raschig Rings Coolant - dry ice/acetone	-65
8	10	60	9.5	As above	-7	As above	-7	As above	-65
9	10	60	1.6	As above	-7	As above	-7	As above	-65
10	1	60	1.6	As above	-7	As above	-7	As above	-65
11	2.5	60	1.6	As above	-7	As above	-7	As above	-65
12	10	60	1.6	As above	-7	As above	-7	Graham condenser coolant 100% methanol(1)	-55

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TABLE 2 - Continued

TEST	INITIAL ACETONE CONCENTRATION (w/v)	STRIPPING TIME (min.)	FLOW RATE. L/min.	CONDENSER 1		CONDENSER 2		CONDENSER 3	
				DESCRIPTION	TEMP °C	DESCRIPTION	TEMP °C	DESCRIPTION	TEMP °C
13	10	60	1.6	As above	-7	As above	-7	Double-walled inner coil condenser, coolant 100% methanol(1)	-55
14	10	60	1.6	Double wall, inner coil glass condenser, coolant 25% ethanol	-7	500 mL Kjeldhal flask filled Raschig Rings coolant dry ice/ acetone	-65	Double wall, inner coil glass condenser, coolant 100% methanol	-55
15	1	120	1.6	As above	-7	As above	-65	As above	-55
16	0.1	120	1.6	As above	-7	As above	-65	As above	-55

(1) Coolant chilled by passage through copper coil immersed in acetone/dry ice bath

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The remaining tests (Test 10-16), shown in Table 3, confirmed that the efficiency of the condensation of water vapour and acetone was in general proportional to the gas flow rate, the initial acetone concentration and the temperature of the condensers.

When the starting acetone concentration was reduced to 1% (w/v) the weights of the respective condensates decreased substantially. While the weights of condensates could be determined with ease, the measurement of acetone concentrations and hence the recovery of acetone, were difficult to achieve due to the relatively poor efficiency with which small volumes of solution could be recovered quantitatively from more complex condensers such as the 500 mL Kjeldhal flask filled with Raschig rings. For mass balance purposes, the data are therefore incomplete but those shown for selected tests (Table 4) indicate that the majority of the acetone was detected in the condensers cooled to -65^{o}C.

An additional source of potential error was the tendency for acetone/water vapour mixtures to condense and freeze on to the inner surface of condensers cooled to subzero temperatures. The goal was to maintain the condenser temperature such that the acetone concentration in the condensate would depress the melting point and hence prevent freeze-up at temperatures as low as -7^{o}C.

The condensers used in this study (Table 2), were selected so as to afford the maximum possible surface area coupled with the lowest possible temperature. The 500 mL Kjeldhal flask filled with Raschig rings afforded a high surface area and the lowest temperatures achieved was that of the dry ice/acetone mixture (approx. -65^{o}C). Based on these preliminary data, two glass condensers were custom manufactured.

Final tests were performed using an acetone/butanol/ethanol solvent mixture similar to that expected in fermenter broths.

TABLE 3: STRIPPING TESTS - ACETONE-WEIGHTS OF CONDENSATES

TEST	INITIAL ACETONE CONCENTRATION (% w/v)	STRIPPING TIME (min.)	FLOW RATE (L/min)	WEIGHT OF CONDENSATE (g)					TIME (min) TO PPT IN DNPH BUBBLER
				2°C (C1)	-7°C (C1)	-7°C (C2)	-65°C (C2)	-55°C (C3)	-65°C (C3)	
1	10	60	19.2	16.6			48			4
2	10	60	19.2	23.0			133			
3	10	60	19.2		38.0		64.5			10
4	1	60	19.2		14.2		10.9			7
5	2.5	60	19.2		16.6		21.7			5
6	5	60	19.2		20.1		32.8			5
7	10	60	19.2		47.7	1.3			63.7	5
8	10	60	9.5		29.5	1.2			26.8	20
9	10	60	1.6		10.8	0.2			10.1	15
10	1	60	1.6		1.3	ND(1)			3.9	20
11	2.5	60	1.6		3.0	ND			7.0	30
12	10	60	1.6		13.1	1.9		7.4		5
13	10	60	1.6		10.2	3.3		11.9		10
14	10	60	1.6		11.7		10.5	0.6		15
15	1	120	1.6		2.9		4.4	0.1		60
16	0.1	120	1.6		2.3		0.7	0.1		60

1. none detected

TABLE 4: CONDENSATION OF ACETONE FROM RECYCLED GAS STREAM(1)

TEST	ACETONE CONCENTRATION IN TEST SOLUTION (g/L)		CONDENSATE 1		CONDENSATE 2		CONDENSATE 3		CONDENSATE 4 (Bubbler)
	START	FINISH	VOL. mL	ACETONE g	VOL. mL	ACETONE g	VOL. mL	ACETONE g	ACETONE g
10	10	11.6	~1.5	NA (2)	ND (3)		4.0	3.74	ND
11	25	24.2	~3.0	NA	ND		9.5	9.21	ND
12	100	89.6	13.5		ND		10.1	9.55	

(1) Test Solution - 1050 mL - 40°C
Flow Rate - 1.6 L/min. (1.52 VVM)

(2) NA - not analyzed

(3) ND - none detected

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6.3.2 Tests of Condenser Efficiencies With Respect to Recovery of Acetone, Butanol and Ethanol From Mixed Vapour Phase

These tests used a butanol, acetone, and ethanol mixture similar in composition to that expected in fermenter broths. The starting solvent concentrations in the starting solution were 6 g/L, 3 g/L and 1 g/L respectively for butanol, acetone and ethanol. The schematic for the gas-stripping system used for these tests is shown in Figure 4. Tests were conducted for 4-6 hours at 1.58 VVM in order to generate sufficient volumes of condensates for analyses. Different combinations of condenser temperatures were employed (Table 5) and the recovery of each solvent was measured (Table 6).

The percentage recovery (% by wt.) for each solvent was also calculated (Tables 7 - 9). The data (Table 7) verify that at the typical volumetric flow rates used in the fermenter-purifier, acetone requires a low temperature for efficient recovery. Liquid nitrogen (estimated temperature -200^{o}C) was the most effective coolant and recovery of acetone was negligible at temperatures as low as -40^{o}C. The data for acetone will be biased due to the high probability that the solvent condensing out at higher coolant temperatures will be re-entrained in the vapour phase and hence migrate to the -200^{o}C condenser. A significant percentage (4-5%) of the acetone was found to be trapped in the water in the gas washing bottle following the liquid nitrogen cooled condenser. The data for Test 2 (Tables 6 - 8) suggest that a leak was present in the system hence the recovery of acetone was low.

It is apparent that the acetone is poorly recovered at coolant temperatures which can be more readily utilized in the process design. Butanol, on the other hand, can be recovered at -7^{o}C (Table 8) with an efficiency between 73 and 90% of the total amount of butanol that was

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TABLE 5: STRIPPING TEST - MIXED SOLVENTS/DESCRIPTION OF CONDENSERS

TEST	BUTANOL/ ACETONE/ ETHANOL g/L	STRIPPING TIME (min.)	FLOW RATE (L/min)	CONDENSER 1 DESCRIPTION	CONDENSER 1 TEMP °C	CONDENSER 2 DESCRIPTION	CONDENSER 2 TEMP °C	CONDENSER 3 DESCRIPTION	CONDENSER 3 TEMP °C	CONDENSER 4 DESCRIPTION	CONDENSER 4 TEMP°C
1	6:3:1	300	1.6	Double walled Coil glass Condenser Coolant - 25% Ethanol	-7	Double-wall coil conden-ser, Coolant Methanol	-55	500 ml Kjeldhal Flask filled with Raschig Rings, Coolant Dry Ice/Acetone	-65	Cold thimble Coolant Liquid N_2	-200
2	6:3:1	360	1.6	As above	-7	As above	-55	As above	-65	As above	-200
3	6:3:1	300	1.6	As above	-7	Double wall inner coil, long path Coolant - Methanol	-55	Cold thimble Coolant - Liquid N_2	-200		
4	6:3:1	360	1.6	As above	-2	As above	-40	As above	-200		

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TABLE 6: STRIPPING TESTS - MIXED SOLVENTS/SOLVENT RECOVERY DATA

TEST	REACTOR SOLUTION (g/L)					SOLVENTS RECOVERED (g) [1]																					
	START			FINISH		-2°C (C1)			-7°C (C1)			-40°C (C2)			-55°C (C2)			-65°C (C2/C3)			-200°C (C2/C3)					
	B	A	E(2)	B	A	E	B	A	E	B	A	E	B	A	E	B	A	E	B	A	E	B	A	E	B	A	E
1	6.46	3.40	1.04	4.44	0.95	0.82				1.45	0.06	0.08				ND(3)	ND	ND	0.16	1.62	0.04				0.01	0.41	ND
2	6.52	4.07	1.01	3.90	0.89	0.82				1.58	0.06	0.06				ND	ND	ND	0.18	0.21	0.06				0.04	0.57	0.01
3	6.43	3.93	1.09	4.76	1.22	0.92				1.31	0.09	0.05				0.22	0.51	0.05							0.26	1.72	0.07
4	6.42	3.77	1.21	4.32	0.96	0.87	0.99	0.10	0.07				0.89	0.04	0.08										0.03	2.20	0.05

(1) Acetone was recovered in water in gas washing bottle following the -200°C condenser.
Test 1 - 0.108 g
Test 2 - 0.081 g
Test 3 - 0.144 g
Test 4 - 0.135 g

(2) A = Acetone
B = Butanol
C = Ethanol

(3) none detected

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TABLE 7: STRIPPING TESTS - MIXED SOLVENTS/CONDENSATION OF ACETONE(1)

TEST	CONDENSATES (wt %)							OVERALL RECOVERY %
	-2°C (C1)	-7°C (C1)	-40°C (C2)	-55°C (C2)	-65°C (C2/C3)	-200°C (C3/C4)	GAS WASHING BOTTLE	
1		3.4		ND(2)	66.1	16.7	4.4	89.7
2		1.9		ND	6.6	17.9	2.6	26.4
3		3.3		18.8		63.5	5.3	90.9
4	3.6		1.4			78.3	4.8	83.3

(1) Expressed as % by weight of total weight of acetone that was stripped
(2) none detected

TABLE 8: STRIPPING TESTS - MIXED SOLVENTS/CONDENSATION OF BUTANOL(1)

TEST	CONDENSATES, (wt.%)						OVERALL RECOVERY, %
	-2°C (C1)	-7°C (C1)	-40°C (C2)	-55°C (C2)	-65°C (C2/C3)	-200°C (C3/C4)	
1		71.8		ND(2)	7.9	0.5	80.2
2		60.3		ND	6.9	1.5	68.7
3		78.4		13.2		15.6	107
4	47.1		42.4			1.4	91.0

(1) Expressed as % by weight of total weight of butanol that was stripped.
(2) none detected

TABLE 9: STRIPPING TESTS - MIXED SOLVENTS/CONDENSATION OF ETHANOL(1)

TEST	CONDENSATES (wt.%)						OVERALL RECOVERY, %
	-2°C (C1)	-7°C (C1)	-40°C (C2)	-55°C (C2)	-65°C (C2/C3)	-200°C (C3/C4)	
1		36.3		ND	18.2	ND	54.5
2		31.6		ND	31.6	5.3	68.4
3		29.4		29.4		41.2	100
4	20.6		23.5			14.7	58.8

1. Express as % by weight of total weight of ethanol that was stripped
2. Not determined

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recovered. In Test 2, the overall recovery of butanol was lower than in the other tests, which again suggests the possibility of a leak in the system.

The recovery of ethanol was generally lower than for either the butanol or acetone (Table 9). This is attributed in part to the poorer accuracy and precision of the analytical methodology at the lower concentrations observed with ethanol.

An interesting observation with all three solvents was the significant increase in the solvents recovered at $-55^{o}C$ in Test 3 when compared with Tests 1 and 2. Test 3 was the first time that the new long path glass condenser was used and it appeared to offer a significant improvement in the condensation efficiency compared with that of the shorter double- walled condensers used in earlier tests.

Based on these data, it was decided to use the two long path condensers in the recycle gas train and to cool the first condenser to approximately $-7^{o}C$ in order to remove most of the water vapour together with a small amount of co-condensed butanol. The second condenser would be cooled by coolant recycled from a Neslab recirculating cooler system (Model RTE 5) set at the lowest possible temperature ($-20^{o}C$). The lowest temperature observed in use was $-16^{o}C$.

It was recognized that at the temperatures used with the twin condensers, the recovery of acetone would be poor. This prompted investigations on alternative recovery systems for the entrained solvents in the gas recycle phase. Acetone removal remained the prime concern.

6.3.3 The Recovery of Organic Vapours by Molecular Sieve Adsorbents

Molecular sieves have been used widely to remove water from 95%(w/v) ethanol and hence break the azeotrophic mixture to yield anhydrous ethanol. A unique group of molecular sieve materials have been developed in which modification of the aluminosilicate matrix renders the adsorbent hydrophobic. Such compounds, of which silicalite (Union Carbide Ltd.) is a well known representative, have been demonstrated to selectively recover organic molecules from aqueous solutions. Their hydrophobic properties mean that under the recommended protocol for use, the water adsorption capacity is low. The extent of adsorption of water is dependent on the nature of the bonding material used to form the pellets. Thus, silicalite SR-115, the silica bonded product is stated to adsorb less than 1%(w/w) of water while the alumina bonded pellets (SP-115) is said to take up a slightly greater percentage of water. (Union Carbide Technical Information).

It was demonstrated by Milestone and Bibby (1981) that silicalite can concentrate butanol and ethanol from dilute aqueous solution (0.5 - 2% w/v) with the alcohols being recovered by thermal desorption. Using column adsorption tests coupled with thermal desorption, the adsorption of ethanol from a 2% (w/v) solution gave rise to a condensate containing 32% (w/v) ethanol while a 0.5 (w/v) butanol solution yielded a condensate with 98% (w/v) butanol. Butanol was more rapidly and more strongly adsorbed than ethanol but in mixed acetone/butanol mixtures, the acetone interfered with the uptake of the butanol.

Maddox (1983) subsequently showed that butanol could be adsorbed from a fermentation broth but no indication was given as to the uptake of acetone or ethanol. However, Bul *et al.*, (1985) identified silicalite to effectively adsorb ethanol from dilute ethanol/glucose solutions similar in concentration to those encountered in fermentation broths.

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These data, in addition to evidence that organic molecules are also adsorbed from the vapour phase by silicalite (Shultz-Sibbel et al., 1982, Garg and Ausikaitis, 1985) prompted us to investigate the use of silicalite for the recovery of acetone, butanol and ethanol from the fermenter-purifier recycle vapour stream.

The schematic of the system used to undertake the vapour phase stripping/silicalite adsorption tests with the different solvents is shown in Figure 5. The data (Figure 10) show that the uptake of acetone from the vapour phase is rapid with equilibrium between the adsorbent and acetone concentration in the vapour phase, occurring within less than 30 minutes. Conversely, the rate of uptake of ethanol (Figure 11) was much slower with equilibrium still not being attained after even three hours and ten minutes. The uptake of butanol from the vapour phase (Figure 12) was linear with an "elbow" and subsequent equilibrium between adsorbent and butanol in the vapour phase occurring after 1 1/2 hours stripping.

When an aqueous solution of butanol/acetone/ethanol (6:3:1) was gas-stripped at 40^{o}C, the adsorption pattern by the silicalite was much more complex (Figure 13). In keeping with the observations for acetone alone, the concentration of acetone in the starting solution decreased significantly in the first thirty minutes. However, the butanol subsequently displaced the acetone with the plateau for the uptake of butanol being attained after 1 1/2 hours. The acetone concentration in the solution being stripped was higher at the close of the test. This was attributed to loss of both volatiles and moisture causing an apparent concentration of the acetone. The adsorption of ethanol for the mixed vapour phase was slight and as in the case of the acetone, the lower molecular weight compound appeared to be displaced by butanol from the adsorption sites on the silicalite. This tendency for butanol to displace the lower alcohols and acetone is in keeping with published information on the high affinity of silicalite for butanol (Milestone and Bibby, 1981).

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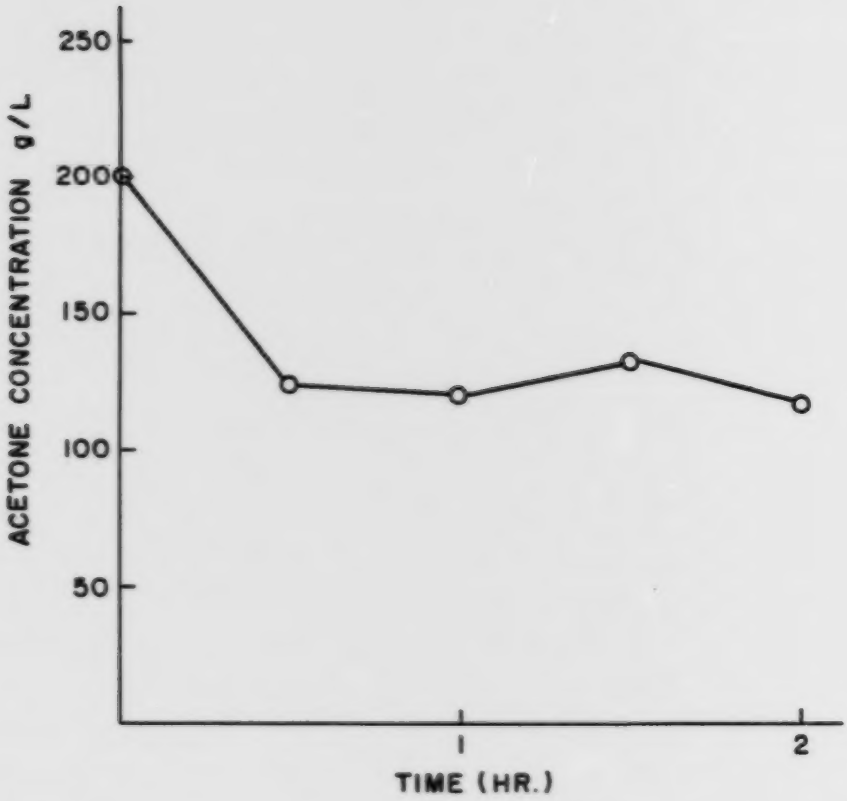

FIGURE 10. ADSORPTION OF ACETONE FROM VAPOUR PHASE BY SILICALITE SR-115(421.3g) AS INDICATED BY THE ACETONE CONCENTRATION OF SOLUTION (200mL) BEING STRIPPED AT 40°c.

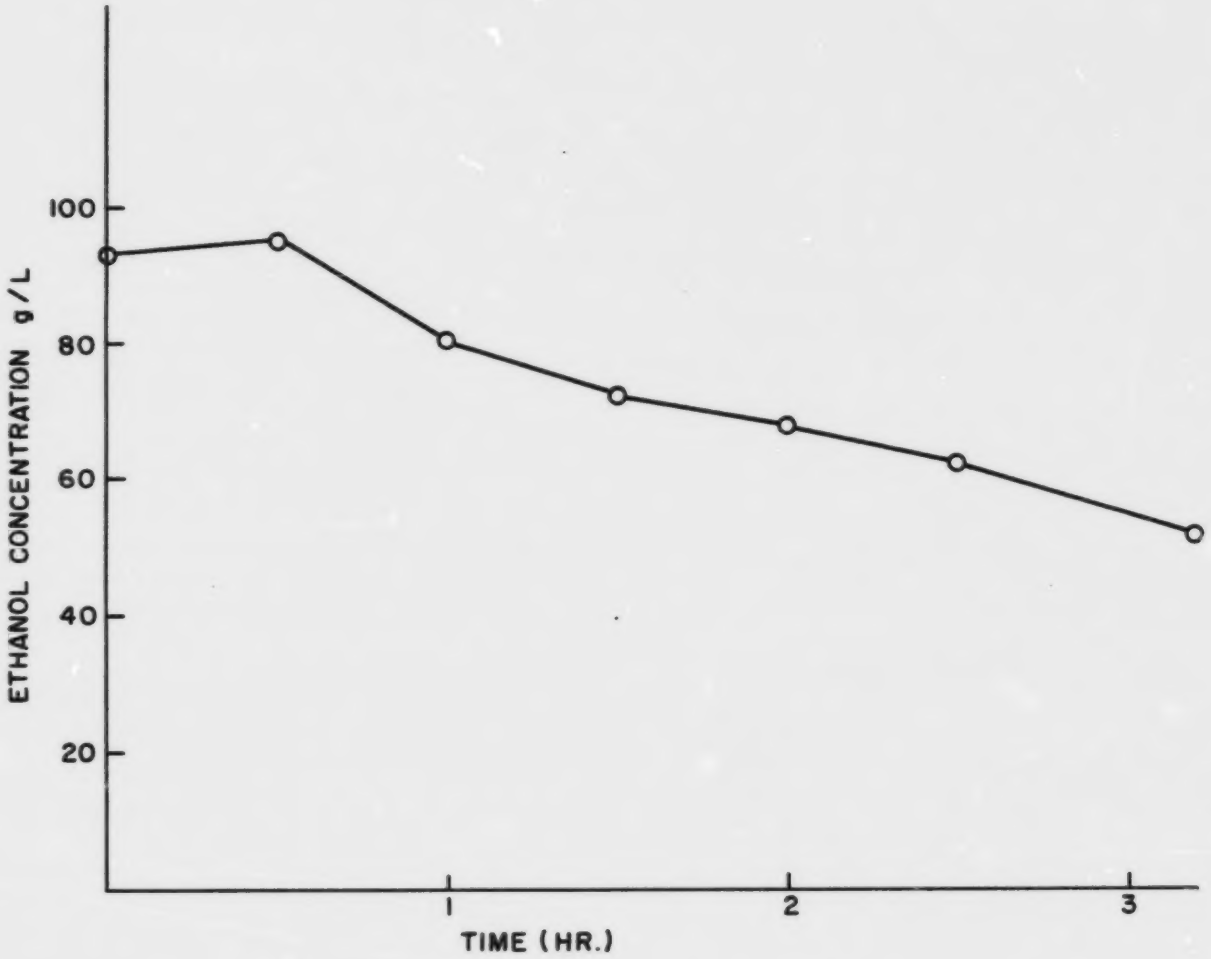

FIGURE 11. ADSORPTION OF ETHANOL FROM VAPOUR PHASE BY SILICALITE SR-115 (421.3g) AS INDICATED BY THE ETHANOL CONCENTRATION OF SOLUTION (300mL) BEING STRIPPED AT 40°c.

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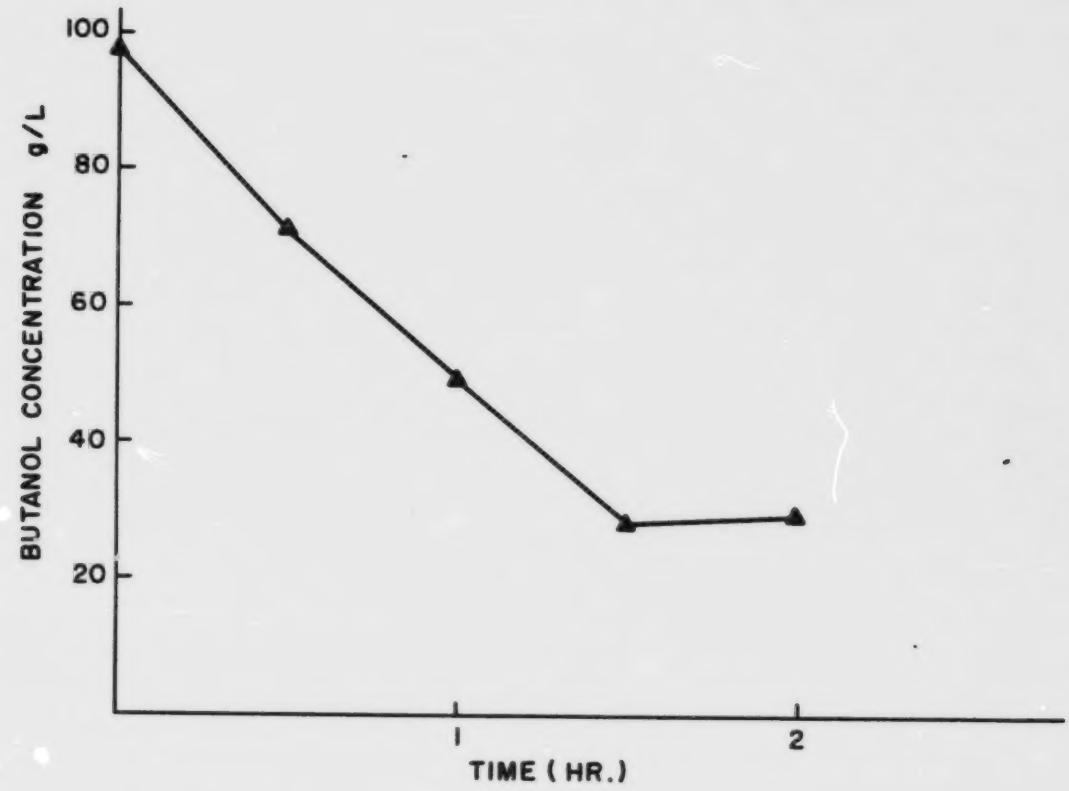

FIGURE 12. ADSORPTION OF BUTANOL FROM VAPOUR PHASE BY SILICALITE. SR-115. (421.3g) AS INDICATED BY THE BUTANOL CONCENTRATION OF SOLUTION (400mL) BEING STRIPPED AT 40°c.

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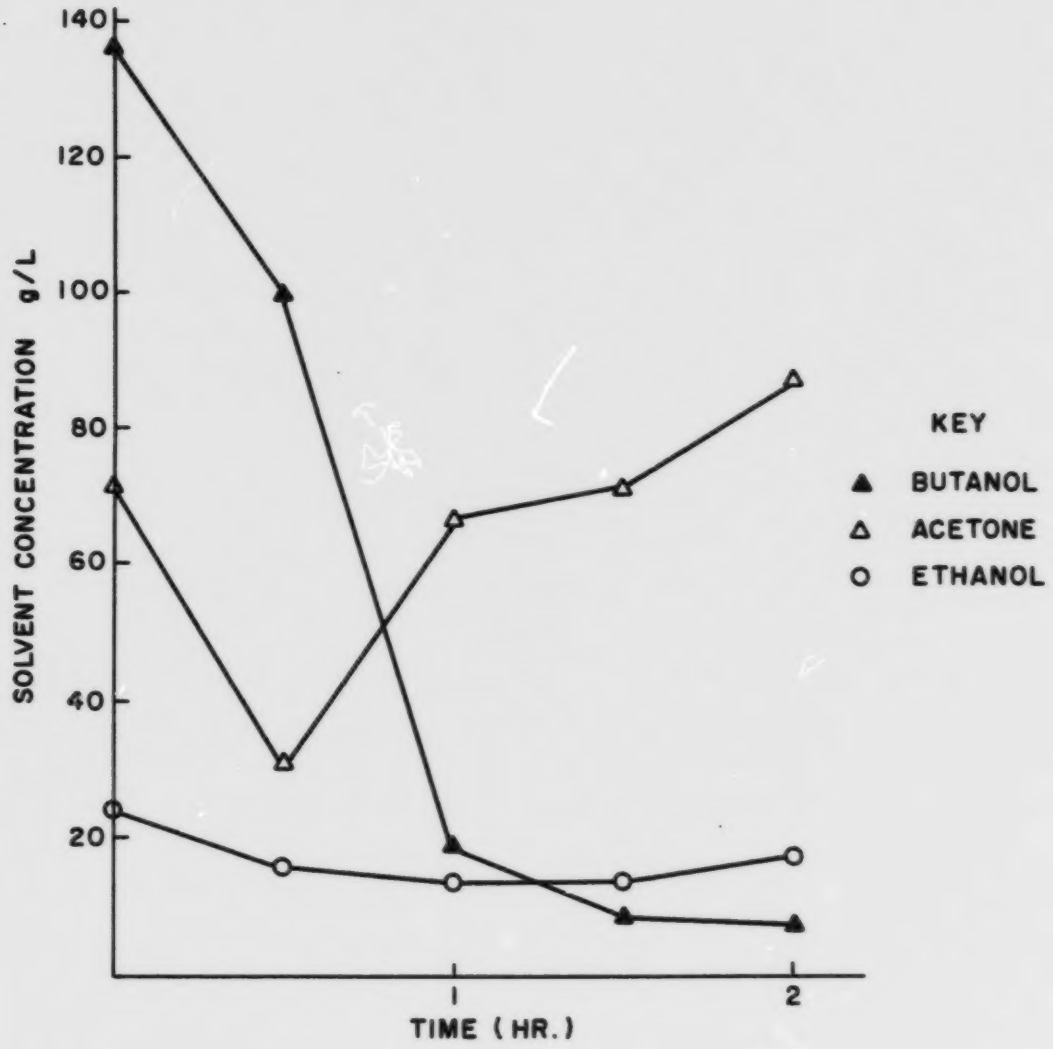

FIGURE 13. ADSORPTION OF MIXED SOLVENTS FROM VAPOUR PHASE BY SILICALITE SR-115 (421.3g) AS INDICATED BY THE SOLVENT CONCENTRATION OF SOLUTION (200mL) BEING STRIPPED AT 40°c. BUTANOL / ACETONE / ETHANOL – 6 : 3 : 1 AT START.

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Based on the loss of solvent from the solutions being stripped, the maximum adsorption capacities for the solvents acetone, butanol and ethanol when stripped from pure solutions were calculated to be 4.20 g/100 g, 7.27 g/100 g and 5.13 g/100 g, respectively of silicalite.

The data for the recovery of solvents by thermal desorption indicate that the efficiencies of the thermal desorption of acetone, butanol and ethanol respectively, from the solvent saturated silicalite were generally poor (Table 10). In this test, the yield of recovered acetone was significantly higher than the loading calculated from experimental data. There appeared to be a significant memory effect from the previous test. These low recovery figures and memory effects are attributed to the geometry of the silicalite column (diameter 5.5 cm) being unsuitable for desorption based on the use of external heating pads. Thus, the temperature of the total bed was not being elevated to the desired temperature for desorption, i.e., cold zones were probably present in the centre of the bed.

Nevertheless, the above data verified the ability of silicalite to adsorb butanol, acetone and ethanol from the vapour phase. More detailed investigations on the optimization of the desorption cycle were not justified at the present time, but for inclusion of the silicalite as a vapour phase adsorbent for active fermentations, the column geometry was modified significantly (2.5 x 30 cm) and the loading of silicalite was also much less (153 g). This was intended to assure more uniform temperatures across the bed during the thermal desorption with nitrogen stripping gas.

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TABLE 10: RECOVERY OF SOLVENTS FROM SILICALITE SR-115[1]

TEST	STARTING SOLUTION	ACETONE			BUTANOL			ETHANOL		
		LOADING (g)	WT. DESORBED	% RECOVERY	LOADING (g)	WT. DESORBED	% RECOVERY	LOADING (g)	WT. DESROBED	% RECOVERY
1	Acetone - 200 g/L	17.68	14.33	81.1						
2	Butanol/Acetone/Ethanol 12 6 2 g/L	1.13	14.35		26.15	5.51	21.1	2.30	1.18	51.3
3	Butanol - 100 g/L				30.61	12.17	39.8			
4	Ethanol - 100 g/L							21.62	16.88	78.1
5	Ethanol - 100 g/L							10.2	4.49	44.0

(1) 421.3 g Silicalite SR-115
Heat applied via heating pads around colume + nitrogen flow to displace solvents

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6.4 Fermentations

6.4.1 Preliminary Test

The initial fermentation in the fermenter-purifier (PFO) yielded interesting but somewhat anomolous data (Figure 9). Shortly after the start of the fermentation (Day 2) the acetone concentration in the broth was greater than the butanol concentration. Similarly, in the later stages (approximately Day 25), a sharp increase was observed in the acetone concentration of the broth (6 - 7 g/L) while the butanol concentration remained relatively low. These elevated concentrations were not matched by equivalent peaks being detected in the condensates collected at 7°C during these time intervals. By way of comparison, the increase in the butanol and acetone concentration of the broth from Day 10 - 11 was matched by an equivalent, more highly concentrated solution in the condensate. The discrepancy, therefore, was believed to be due to the low efficiency of the condensation of acetone at a temperature of 7°C. This deduction led to our efforts towards improving the condenser system. However, HPLC data obtained at a later date identified that the elevated acetone concentrations in the broth may have been butyric acid, since acetone and butyric acid elute at identical retention times on the HPLC column used in this test. A test confirmed that a low concentration of butyric acid may be stripped from aqueous solutions at 40°C but that compared with the solvents being investigated, the butyric acid concentration would have to be very high to cause a significant amount of butyric acid in the condensate. Thus, it may be assumed that acetone is the major constituent in the condensates. In the case of the fermentation broths, it is not possible to estimate how much of the perceived acetone peak (6 - 7 g/L) at Day 25 was actually butyric acid. However, if the butyric acid concentration was greater than 4 g/L it would be toxic towards the Cl. acetobutylicum. The sharp decrease from Day 25 - 26 infers however that either the

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compound was actively metabolized by the cells or lost by volatilization. Since no increase was subsequently noted in the butanol concentration (the end product of butyric acid metabolism), the possibility exists that the peak in the broth at Day 25 was due in part to acetone accumulation.

This preliminary fermentation served, therefore, to guide the subsequent direction of the program. Irrespective of the anomolies noted above, the butanol concentration in the broth was generally low and production could not be regarded as continuous.

6.4.2 Sterilization

Following fermentation PFO and completion of the tests on condenser systems, the circuit for the fermenter-purifier was modified to include the two long-path glass condensers. At the typical operating pH of the ABE fermentation, contamination was expected to be a major concern, particularly in view of the difficulty faced in sterilizing the total system.

Sterilization procedures, other than moist heat, were assessed. These included the sparging of ozone through sterile water in the fermenter with the ozone gas being allowed to permeate and vent through both condensers and all tubing in the circuit. Tests verified that ozone was reaching all sections of the circuit and a contact time of 3 hours was used. Following the ozonation stage, the fermenter-purifier system was purged extensively with filter sterilized (0.22 μm) nitrogen gas. The fermenter was then charged with sterile reinforced clostridial medium (RCM) and the gas recycle started at room temperature (23°C). A heavy bacterial growth developed in the fermenter within 36 hours. Thus, the sterilization by ozonation was not effective.

Subsequent to this test, the fermenter-purifier circuit was modified so that all fittings and tubing associated with the fermenter could be sterilized by autoclaving. This included the tubing between the gas recycle pump and the fermenter, all reservoirs and tubing supplying nutrients, make-up water etc., all tubing between the fermenter and the first condenser and of course, the fermenter itself.

6.4.3 Preliminary Observations

(a) Initiation of Solventogenesis

Preliminary tests (not documented in detail) were performed with both the redesigned fermenter-purifier and also the BRL airlift fermenter. Considerable difficulty was experienced in achieving initiation of solventogensis. Two factors which may have contributed to this problem are the pH and the effect of agitation due to the gas recycle. Initial tests with the fermenter-purifier involved starting the gas recycle immediately after the culture had been inoculated and the system purged with sterile, oxygen-free nitrogen. In these cultures, the pH was not controlled. Accordingly, a decrease in pH was noted but the expected transition to the solventogenic phase did not materialize. The pH value tended to stay very low (e.g., 3.5).

The chemically defined medium used in these tests did not appear to afford the ingredients required to buffer the pH within the optimal range for solvent production as is noted with media based on molasses and organic nitrogen supplements. Therefore, pH control was used in subsequent tests. The effect of this lack of pH control may have been compounded by the use of gas recycle immediately after inoculation of the fermentation. It has been demonstrated that vigorous agitation reduced significantly the yield of solvents in batch cultures (Yerushalmi and Volesky, 1985, Doremus et al., 1985). This is attributed to the

agitation causing a reduction in the concentration of the dissolved hydrogen in the fermentation broth (Doremus et al., 1985). Thus, fermentations operated under pressure to create hydrogen/ carbon dioxide, partial pressures in the headspace of the fermentation and hence maintain the concentration of the gases dissolved in the broth, did not display the same effects of agitation on solvent production (Doremus et al., 1985) and in fact butanol production was enhanced.

It is suspected therefore, that the early difficulties with the acetone/ butanol fermentation were largely attributable to the effects of the gas re-cycle on the initiation of the solventogenic stage. In some preliminary tests, acetone and/or butanol were detected but the levels were low. Furthermore, the time required for solvent production both in these tests and in subsequent fermentations were much longer than expected for a quiescent batch culture.

Failure to initiate solvent production leads to continued production of organic acids and without pH control, the risk of toxic levels of free acids being generated will be high. Thus, whereas either factor may individually cause these early difficulties in even batch cultures, it is suspected that the unique characteristics of the gas recycle concept served to compound the situation.

(b) Culture Contamination or Morphological Changes

A related difficulty was caused by the morphological changes occurring in the cultures with age. At some stages, as many as five cell morphologies would be seen in a culture, so much so, that contamination was suspected. This led to several fermentations being halted and this action may have been precipitous on our part. Morphological changes have been associated with solvent production and Spivey (1978) described several expected morphologies for Cl. acetobutylicum in molasses medium

at different operating conditions. Jones et al., (1982) correlated solvent production with morphological changes occurring in Cl. acetobutylicum cultures in an industrial fermentation medium. Their observations suggested that a clostridial form was involved in the production of neutral solvents from the organic acids. Subsequently, Long et al., (1984) with a defined chemical medium, identified that the morphological changes and initiation of solvent production were related to the inhibition of cell division due to critical levels of organic acids accumulating in the culture medium. The unique culture environment of our cultures involving the gas recycle may introduce the possibility of yet more complex morphological transitions. At the same time, contamination must be regarded as an ever present risk and therefore, without undertaking more detailed tests on such cultures, it is difficult to ascertain whether the different morphological forms are Cl. acetobutylicum cells or contaminants. In some instances, however, the presence of contamination was easily identified due to the non-characteristic variations in cell morphologies.

6.4.4 Fermentation PF1

(a) General Observations

This fermentation was the first of the more positive tests. Based on the information derived from previous tests, the fermentation was initially allowed to proceed in batch mode with only a slight flow of oxygen- free nitrogen (0.4 L/min.) being used to gently agitate the contents of the fermenter and maintain anaerobic conditions. The pH was maintained by the addition of sterile sodium hydroxide as required (manual addition).

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The data for PF1 are shown in Figures 14 and 15, Tables 11 and 12. When in the batch stage, a rapid decrease in pH was noted corresponding to the acidogenic stage of the fermentation. By maintaining the pH at 4.5 - 4.7, the culture was enabled to switch into the solvent producing mode. The beginning of the vapour phase stripping and gas recycle was timed to coincide with the phase of most rapid butanol production. Thus, gas recycle was commenced at Day 7.

The continuous vapour stripping rate (1.6 L/min = 1.52 VVM) brought about a sharp decline in the butanol concentration of the broth over an interval of five days. It appeared therefore, that the use of vapour stripping led to initially, a reduction and then an eventual loss of butanol formation in the culture. A point of note (Figure 14) is that the solvent production did not totally stop and the acetone concentration in the broth peaked two days after the butanol concentration had reached its maximum. These data for acetone concentrations in broth samples were determined by gas chromatography hence interferences, such as butyric acid were ruled out. However, these fluctuations meant that the butanol/acetone/ ethanol ratio varied widely over the course of the fermentation (Table 11). The ethanol maximum concentration in the broth also coincided with that for acetone.

When the gas recycle rate was reduced to 0.4 L/min. on Day 17 to counteract the apparent detrimental effects of the 1.52 VVM stripping rate, a slight increase resulted in the butanol concentration of the broth. However, upon increasing the gas recycle rate to 0.76 VVM (0.8 L/min.) the butanol concentration decreased to almost zero, at which time the fermentation was stopped.

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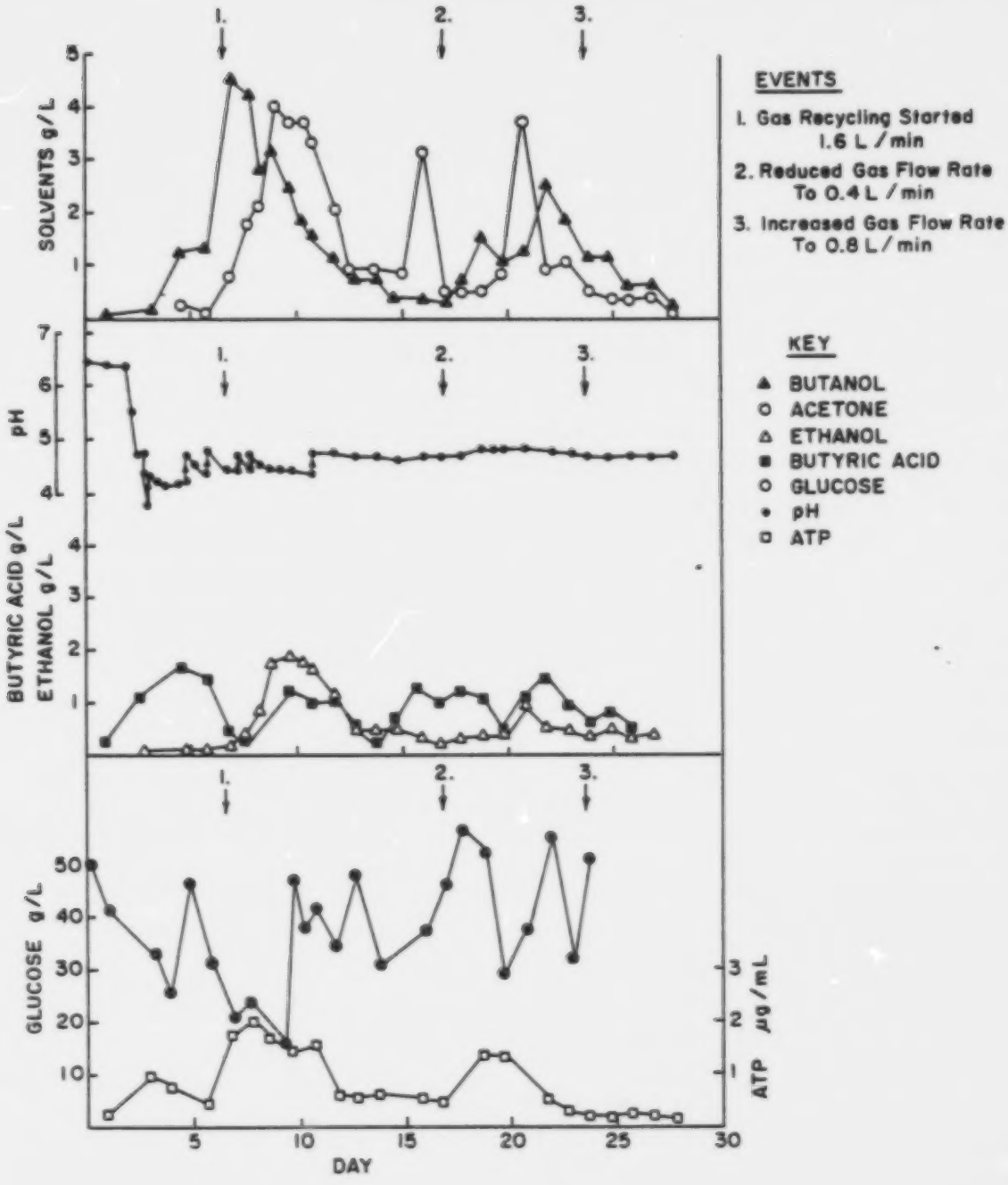

FIGURE 14. FERMENTATION PFI – FERMENTER-PURIFIER. ANALYSES OF BROTH.

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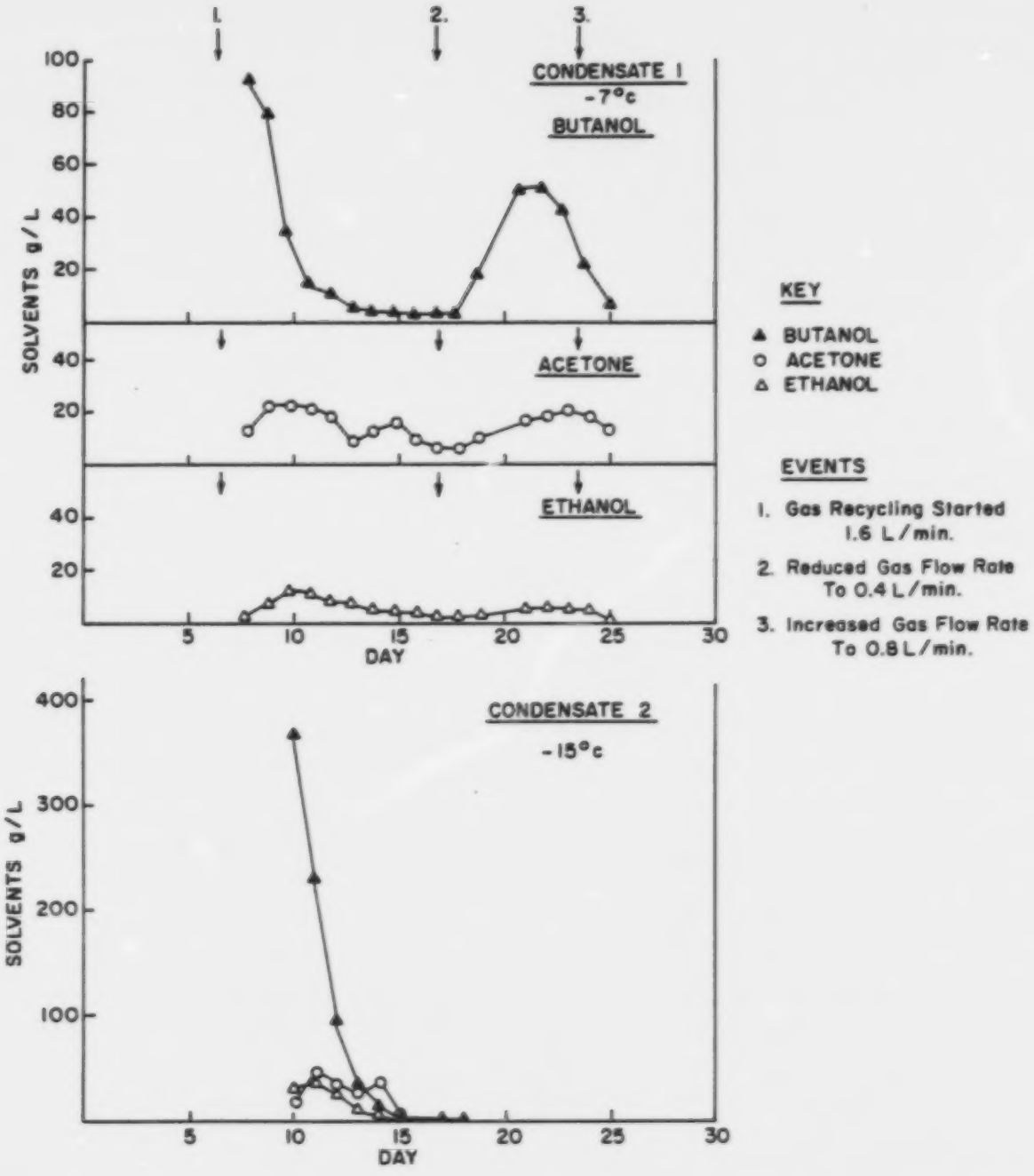

FIGURE 15. FERMENTATION PFI – FERMENTER-PURIFIER. SOLVENT CONCENTRATIONS IN CONDENSATES.

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TABLE 11: FERMENTATION PFL - FERMENTER-PURIFIER/MONITORING OF BROTH

DAY	pH(1)	ATP ng/mL	GLUCOSE (2)	CONCENTRATION, g/L							SOLVENT RATIO (3)	COMMENTS
				ACIDS				SOLVENTS				
				ACETIC	ISO-BUTYRIC	BUTYRIC	CAPROIC	BUTANOL	ACETONE	ETHANOL		
0	6.45											
1	6.4	298	41.8			0.22	0.24		0.003	0.02		
2	6.3											
3	4.3	959	33.2	0.07	0.02	1.04	0.04	0.106	0.073	0.033		
4	4.2	749	25.6									
5	4.5		46.3	0.01		1.58	0.34	1.23	0.23	0.067	18:3:1	
6	4.8	450	30.9	0.02		1.49	0.21	1.32	0.061	0.095	22:1:1	Gas Recycle Started 1.6 L/min.
7	4.6	1750	21.1	0.22		0.41	0.02	4.57	0.79	0.194	22:4:1	
8	4.5	2000	23.6		0.04	0.36	0.04	4.25	1.79	0.399	10:4:1	
8.5								2.84	2.12	0.88	4:2:1	
9	4.4	1700	15.8					3.18	4.0	1.77		
10	4.4	1425	46.9			1.28	0.57	2.48	3.8	1.81		
11	4.7	1500	41.7		0.42	0.90	0.59	1.60	3.31	1.58	1:2:1	
12	4.7	575	34.2	0.12	0.22	1.09	0.65	1.12	2.02	1.13	1:2:1	
13	4.7	575	47.3			0.52	0.12	0.71	0.913	0.478	1.5:2:1	
14	4.7	600	30.7		0.03	0.35		0.78	0.90	0.45	1.5:2:1	
15	4.6				0.06	0.66		0.4	0.87	0.45		
16	4.65	500	37.1			1.26		0.4	3.15	0.32		
17	4.65	475	45.7			0.96		0.4	0.46	0.26		
18	4.7		57.1			1.16		0.72	0.58	0.34	2:1.5:1	Flow rate reduced to 0.4 L/min.

1. pH before adjustment
2. Glucose concentration (g/L) prior to adding additional glucose
3. Solvent ratios rounded off

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TABLE 11 - Continued

DAY	pH(1)	ATP ng/mL	GLUCOSE (2)	CONCENTRATION, g/L ACIDS ACETIC	ISO-BUTYRIC	BUTYRIC	CAPROIC	SOLVENTS BUTANOL	ACETONE	ETHANOL	SOLVENT RATIO (3)	COMMENTS
19	4.8	1350	52.0			1.01		1.54	0.53	0.37	4:1.5:1	
20	4.8	1300	28.9		0.10	0.42		1.11	0.84	0.35	3:2.5:1	
21	4.85		36.8			1.02		1.22	3.70	0.91	1:4:1	
22	4.8	450	56.3	0.74		1.41		2.52	0.97	0.50	5:2:1	
23	4.7	290	31.6			0.96		1.89	1.08	0.47	4:2:1	
24	4.7	130	51.2		0.10	0.52		1.61	0.48	0.41	4:1:1	Flow rate increased to 0.8 L/min.
25	4.7	125	48.8	1.01	0.23	0.71		0.62	0.35	0.30	2:1:1	
26	4.65	225	142		0.11	0.49		0.62	0.35	0.30	2:1:1	
27	4.7	180	116					0.67	0.45	0.38	2:1:1	
28	4.7	110	76.2									

1. See Table 10
2. See Table 10
3. See Table 10

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(b) Solvent Recovery

Butyric acid concentrations fluctuated during the course of the fermentation. Other acids identified in the broth at different sampling times were acetic, isobutyric and caproic acids. The caproic acid was only seen at early stages of the fermentation. Glucose consumption at that time was also generally high. During the remainder of the fermentation, the daily consumption of glucose was erratic and did not follow any well defined pattern. The metabolic activity of the cells as indicated by the ATP levels reached plateaus at periods of relatively high solvent producing activity.

Late in the fermentation (approximately Day 21), it was identified that the ammonium concentration of the chemically defined broth was close to zero. Sterile ammonium sulphate solution was injected into the broth to maintain a suitable ammonium concentration but this failed to stimulate the culture. Similarly, injecting sterile butyric acid (approximately 5 g/L) failed to restore solvent generation.

This test confirmed that the vapour phase stripping of solvents, particularly butanol, from active fermentations of Cl. acetobutylicum was feasible. Thus, in the first condensate ($-7^{o}C$) (Table 12), the butanol concentration represented typically a concentration factor of x 20 relative to the concentration in the broth. As expected, Condenser #1 removed most of the water vapour in the vapour phase, so that the measured butanol concentrations in the equivalent samples collected from the second condenser ($-16^{o}C$) were significantly higher. A close correlation existed between the concentrations of butanol in the broth and the condensates respectively. The pattern was not so marked in the case of the ethanol and acetone.

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TABLE 12: FERMENTATION PF1 - FERMENTER-PURIFIER/SOLVENT RECOVERY DATA

DAY	CONDENSATE 1 (-7°C) VOLUME (mL)	CONCENTRATION, g/L BUTANOL	ACETONE	ETHANOL	CONDENSATE 2 (-16°C) VOLUME (mL)	CONCENTRATION, g/L BUTANOL	ACETONE	ETHANOL	SOLVENT RECOVERED (g) CONDENSATE 1 + CONDENSATE 2 BUTANOL	ACETONE	ETHANOL	TOTAL	OVERALL SOLVENT RATIO	GLUCOSE ADDED (g)
1-7														111
8	45	92.06	13.0	2.90					4.143	0.585	0.131	4.859	31:4.5:1	
9	69	79.13	21.85	7.44					5.460	1.508	0.513	7.481	10.5:3:1	40
10	66	35.17	21.90	11.60	19.5	369.0	17.95	29.9	9.517	1.795	1.349	12.661	7:1.5:1	
11	50	14.75	21.64	10.52	10	230.9	36.9	36.9	3.047	1.536	0.895	5.478	3.5:2:1	10
12	42	10.80	18.06	7.80	3	97.51	31.93	28.34	0.747	0.855	0.413	2.015	2:2:1	20
13	50	5.95	8.88	6.97	3	33.42	25.44	10.7	0.398	0.520	0.381	1.299	1:1.5:1	4
14	55	5.24	12.7	5.46	1	15.8	34.9	14.3	0.304	0.734	0.314	1.352	1:2:1	70
15	42.3	4.49	16.25	4.48	5.5	2.42	5.61	1.50	0.203	0.718	0.198	1.119	1:3.5:1	
16	58.5	3.78	9.12	3.44					0.221	0.534	0.201	0.956	1:3:1	20
17	35.0	3.05	6.59	2.29	4.8	ND(1)	2.97	ND	0.107	0.245	0.080	0.432	1:3:1	
18	11.0	3.40	5.90	1.86	6.2	1.90	3.16	ND	0.049	0.086	0.020	0.155	2.5:4:1	
19	6.3	18.92	9.83	3.13					0.119	0.062	0.020	0.201	6:3:1	
20		NOT COLLECTED												20
21	11.5	50.36	16.78	4.46					0.579	0.193	0.051	0.823	11.5:4:1	
22	6.0	50.95	18.39	5.05					0.306	0.110	0.030	0.446	10:4:1	
23	5.1	43.52	20.35	5.0					0.222	0.104	0.026	0.352	8.5:4:1	20
24	11.0	22.19	18.38	5.17	3.1	362.2			1.512	0.202	0.057	1.771	26.5:3.5:1	
25	21.0	8.60	13.57	ND*					0.181	0.285		0.466	1:1.5:1	11
TOTALS	584.7				56.5				27.115	10.072	4.679	41.866	6:2:1	326

(1) none detected

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(c) Culture Morphology

The bacterial culture was examined at regular intervals under the microscope. The cell morphologies present varied widely over the course of the fermentation and significant contamination was suspected. However, as noted previously, it was possible that some of the unusual cell morphologies were artifacts of the Cl. acetobutylicum cultures arising from the atypical operating conditions of the fermenter-purifier. In this first active fermentation, the addition of alkali, glucose make-up, antifoam was carried out manually, albeit under aseptic technique. Therefore, the risk of contamination was ever present.

(d) Conclusions

This test confirmed that the recovery of solvents, particularly butanol, from active Cl. acetobutylicum cultures was a feasible concept. However, continuous production of solvents at maximum concentrations could not be maintained.

6.4.5 Fermentation AF1

(a) General Observations

This Cl. acetobutylicum fermentation was carried out in the BRL airlift fermenter. Aside from the differences in design criteria noted previously, compared with Fermentation PF1, this fermentation was operated at a much slower stripping rate. This was of necessity rather than by design. Compared with the fermenter-purifier, the sparger in the airlift fermenter generated much finer bubbles which, due to the relatively viscous nature of the Cl. acetobutylicum fermentation broth gave rise to severe foaming problems. Sterile antifoam solution, initially concentrated Antifoam A (Dow Corning), and then a 1-2% (w/v)

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aqueous solution of Antifoam 1520 (Dow Corning), was added as required but failed to prevent loss of broth from the fermenter due to excessive foaming. This foam problem was compounded by the active generation of gases at certain stages of the fermentation.

The experimental data, Figure 16 and Tables 13 and 14, indicate a similar pattern in many respects to that noted in PF1. A higher butanol concentration was attained in the broth (i.e., 9 g/L) and gas recycle was not initiated until the culture had been maintained at the concentrations for one day. As in the case of PF1, the beginning of gas recycle did not immediately change the solvent concentration but within one day the butanol concentration of broth began to decline steadily. However, due presumably to the slower gas recycle rate (0.075 - 0.2 L/min.) ≡ 0.042 - 0.11 VVM) the rate of decrease of butanol concentration was not as rapid as in the case of the fermenter-purifier. As was noted in fermentation PF1, the acetone and ethanol concentrations in the broth also reached maximum levels approximately 2 days behind the butanol peak.

Glucose consumption was again greatest at the period of maximum rate of butanol production. Thereafter, the pattern was erratic with anomolous readings being observed from Day 20 - 30 when the solvent production was essentially nil. No immediate explanation is available for those very high and very erratic glucose concentrations. They were associated with marked changes in the viscosity of the fermentation broth but do not correlate with significant metabolic activity, as indicated by the ATP concentration. It may be speculated that the high glucose levels are related to the degradation of an exopolymer.

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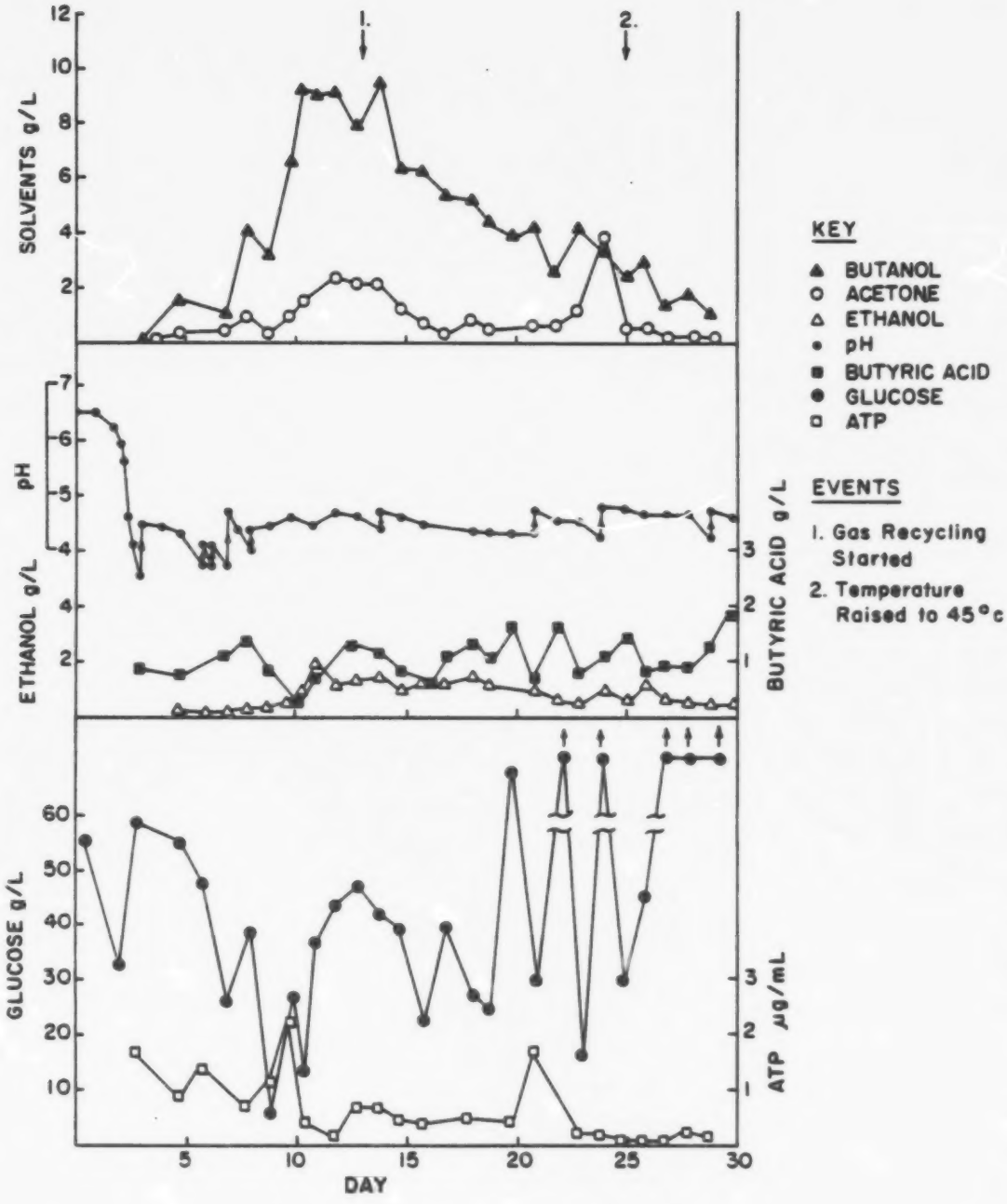

FIGURE 16. FERMENTATION AFI – AIR LIFT-FERMENTER. ANALYSES OF BROTH.

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TABLE 13: FERMENTATION AF1 - AIRLIFT FERMENTER/MONITORING OF BROTH

DAY	pH(1)	ATP ng/mL	GLUCOSE (2)	CONCENTRATION, g/L							SOLVENT RATIO (3)	COMMENTS
				ACIDS				SOLVENTS				
				ACETIC	ISO-BUTYRIC	BUTYRIC	CAPROIC	BUTANOL	ACETONE	ETHANOL		
0	6.5											
1	6.5		55.5									
2	5.6											
3	4.5	1693	58.6	0.042	0.004	0.911	0.137	0.016	0.025	0.048	1:1.5:3	
4	4.4											
5	4.3	892	54.7			0.747	0.343	1.637	0.285	0.060	27:5:1	
6	4.3	1450	47.3									
7	4.4		25.8	0.037	0.002	1.091	0.105	1.088	0.377	0.047	23:8:1	
8	4.4	675	39.0	0.188	0.058	1.322	0.088	3.62	0.881	0.266	13.5:3:1	
9	4.6	1125	5.08		0.003	0.810	0.022	3.42	0.138	0.287	25:1:2	
10	4.6	2200	26.4		0.072	0.245	0.057	6.63	0.993	0.465	14:2:1	
11	4.4	375	36.1			0.875	0.088	9.06	(3.51)4	(2.13)		
12	4.7	150	43.3					9.16	(2.33)	(1.13)		Gas Recycling Started
13	4.6	650	46.7	0.611		1.268	0.608	7.96	2.088	1.23	6.5:2:1	
14	4.7	675	41.3			1.153	0.643	9.53	2.163	1.355	7:1.5:1	
15	4.6	450	39.0	0.001		0.789	0.267	6.28	1.19	0.976	6.5:1:1	
16	4.45	400	22.9			0.46		6.22	0.70	1.10	9:1:1.5	
17			39.4			1.07		5.31	0.35	1.03	15:1:3	
18	4.3	450	27.1			1.26		5.22	0.79	1.38	6.5:1:2	
19	4.3		24.1			1.02		4.46	0.52	1.04	8.5:1:2	

1. See Table 10
2. See Table 10
3. See Table 10
4. Values in parentheses analyzed by HPLC instead of GC (i.e., possibly biased high)

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TABLE 13 - Continued

DAY	pH(1)	ATP ng/mL	GLUCOSE (2)	CONCENTRATION, g/L ACIDS ACETIC	ISO-BUTYRIC	BUTYRIC	CAPROIC	SOLVENTS BUTANOL	ACETONE	ETHANOL	SOLVENT RATIO (3)	COMMENTS
20	4.3		68.1			1.58		3.96	0.51	1.09	3.5:1:2	
21	4.75	400	29.3			0.61		4.22	0.62	0.98	4.5:1:1.5	
22	4.5	1675		0.58		1.56		2.63	0.78	0.60	4.5:1:1	
23	4.5		15.8	0.61		0.73		4.07	1.01	0.53		
24	4.8	130	82.0	0.71		1.03		3.35	3.96	0.83		Temperature raised to 45°
25	4.75	50	29.6		0.40	1.40		2.35	0.45	0.56		
26	4.65	50	45.0	0.02	0.15	0.74		2.83	0.46	1.08		
27	4.65	50	82.0	0.05		0.87		1.21	0.17	0.66		
28	4.65	205	119.0			0.83		1.80	0.08	0.50		

1. See Table 10
2. See Table 10.
3. See Table 10

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TABLE 14: FERMENTATION AF1 - AIRLIFT FERMENTER/SOLVENT RECOVERY DATA

DAY	CONDENSATE 1 (23°C)				CONDENSATE 2 (7°C)				CONDENSATE 3A (1)			
	VOLUME	SOLVENT g/L			VOLUME	SOLVENT g/L			VOLUME	SOLVENT g/L		
	(mL)	BUTANOL	ACETONE	ETHANOL	(mL)	BUTANOL	ACETONE	ETHANOL	(mL)	BUTANOL	ACETONE	ETHANOL
1												
2												
7												
8												
9												
10	36	62.04	5.40	1.79								
11												
13												
14												
15												
17									8(3)	426.3		
19												
21									5.2	563.7		
23												
25	96	23.72	12.44	7.346	103.5	20.4	7.833	4.923				
26									9.7	694.9		
28	48	4.050		2.152	27.0	6.877		2.953	6.1	388.0		41.12
35									6.0	68.96	3.575	2.104
TOTAL	180				130.5				35.0			

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TABLE 14 - Continued

DAY	CONDENSATE 3B (2) VOLUME (mL)	SOLVENT g/L BUTANOL	ACETONE	ETHANOL	SOLVENT RECOVERED (g) BUTANOL	ACETONE	ETHANOL	TOTAL	OVERALL SOLVENT RATIO	GLUCOSE ADDED (g)
1										10
2										30
7										44
8										20
9										85
10					2.233	0.194	0.064	2.491	35:3:1	50
11										25
13										6
14										16
15										20
17	4(3)	159.3	514.0	28.0	4.047	2.056	0.112	6.215	36:18:1	
19										20
21	2	245.9	152.5	10.5	3.422	0.305	0.021	3.748	163:15:1	38
23										63
25					4.390	2.006	1.215	7.611	3.5:2:1	37
26	3.8	274.1	50.97	22.41	7.782	0.194	0.085	8.061	92:2:1	
28	2.5	142.0	325.0	84.07	3.102	0.813	0.393	4.308	8:2:1	
35	6.2	99.84	150.0	4.395	1.033	0.951	0.040	2.024	26:24:1	
TOTAL	18.5				26.009	6.519	1.930	34.458	13.5:3.5:1	464

(1) Solvents desorbed from Silicalite column and recovered by water cooled condenser (7°C)

(2) Solvents desorbed from Silicalite column and recovered by liquid nitrogen cooled condenser

(3) Estimated volume

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(b) Production of Exopolymer

It was noticed in both AF1 and PF1 that as the cultures reached active solvent production phases, the culture media became viscous and required high speed centrifugation coupled with membrane filtration to clarify culture filtrates for analysis of glucose, solvents, acids, etc. A significant role has been proposed for an exopolymer in the metabolism of Cl. acetobutylicum (Haagstrom and Forberg, 1986) and exopolymer production as indicated by viscosity changes was a definite factor in these fermentations. It appeared to be greater in AF1 than PF1, although the different dimensions and operating variables of the two fermenters were obviously important factors. However, the viscosity change in PF1 was particularly noticeable during the phase of low gas recycle (0.4 L/min.) at which time the gas recycle rate was closest to that used throughout with fermentation AF1. Haggstrom and Forberg (1986) identified that production of the expolymer occurred in Cl. acetobutylicum batch cultures during both the growth and acid production stage and when butanol was produced simultaneously from glucose and butyric acid in the medium. Conversely, the concentration of polymer decreased when simultaneous production of butyric acid and butanol was occurring in a culture. These authors hypothesized that the production of exopolymer is the organism's method of "excreting" non-reduced end products of its metabolic cycle as butanol is formed directly from glucose or from readsorbed butyric acid. Alternative, non-reduced end products would be acetic acid or acetone. However, if the driving force for acetic acid or acetone production is low, the polymer production will result. This need by the organism for a sink of non- reduced compounds is due to the excess consumption of carbon (glucose) required to generate the large amounts of reducing power (reduced coenzymes) to achieve the production of butanol.

In our fermentations, the production of the polymer appeared to coincide with the stage of very active acids and butanol production and it was difficult to prevent some loss of sample due to foaming - particularly in the case of the airlift fermenter which had a finer sparger. This factor may be more significant in this system and requires further investigation.

(c) Recovery of Solvents by Silicalite

The use of the silicalite adsorbents generated some very interesting data (Table 14) with thermal desorption yielding condensates (3A) with much higher butanol concentrations than noted with PF1. The majority of the acetone and ethanol were recovered in the nitrogen cooled condenser (3B). However, this is slightly misleading since it was necessary to dilute the 3A condensates with significant volumes of water to obtain homogeneous solutions for analysis. Consequently, any trace amounts of acetone or ethanol in these condensates would be diluted to levels below the detection limit of the HPLC method. Nevertheless, although some solvents may not have been quantitated, the data for 3A and 3B indicate that during the thermal desorption, some fractional distillation is attainable. The solvent ratios in the condensates fluctuated widely reflecting changes in both the solvent concentrations in the broth and the adsorption characteristics of the silicalite relative to the respective solvent concentrations in the vapour phase.

It should be noted that at this stage of the study, the selection of residence time that the vapour phase was in contact with the silicalite was an arbitrary decision.

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6.4.6 Fermentation PF2

(a) General Observations

Fermentation PF2 (Figures 17 and 18 and Tables 15 and 16) was a repeat of PF1 except that a lower rate of gas recycle of 0.8 L/min. (0.66 VVM) was used throughout and Condenser #2 (-16°C) was not operating. The solvent concentration in the broth were also monitored at approximately 12 hour intervals. In this instance, the butanol concentration in the broth continued to increase for one day following the start of the vapour phase stripping. Subsequently, the butanol concentration in the broth declined steadily and at Day 11, when the value was approximately 1.5 g/L, the fermentation was stopped. As occurred in fermentation PF1, the acetone concentration in the broth reached a maximum approximately 48 hrs. after the butanol concentration had peaked. Thus, the acetone was increasing as the butanol concentration was declining.

In this fermentation, the addition of glucose, alkali for pH control, make-up water, was achieved by peristaltic pumps which were controlled manually.

The fermentation foamed badly at Day 2 and some broth was lost into the first receiver flask. Sterile Antifoam 1520 (1% v/v) solution was added as required to control the foaming in the fermentation.

The ATP concentrations were not as expected. Up to Day 5, no significant ATP levels were noted. ATP levels increased as the solvent production was observed and this was accompanied by a period of higher consumption of glucose. Subsequently, as the solvent production declined, the ATP levels were seen to decrease and glucose consumption was very low.

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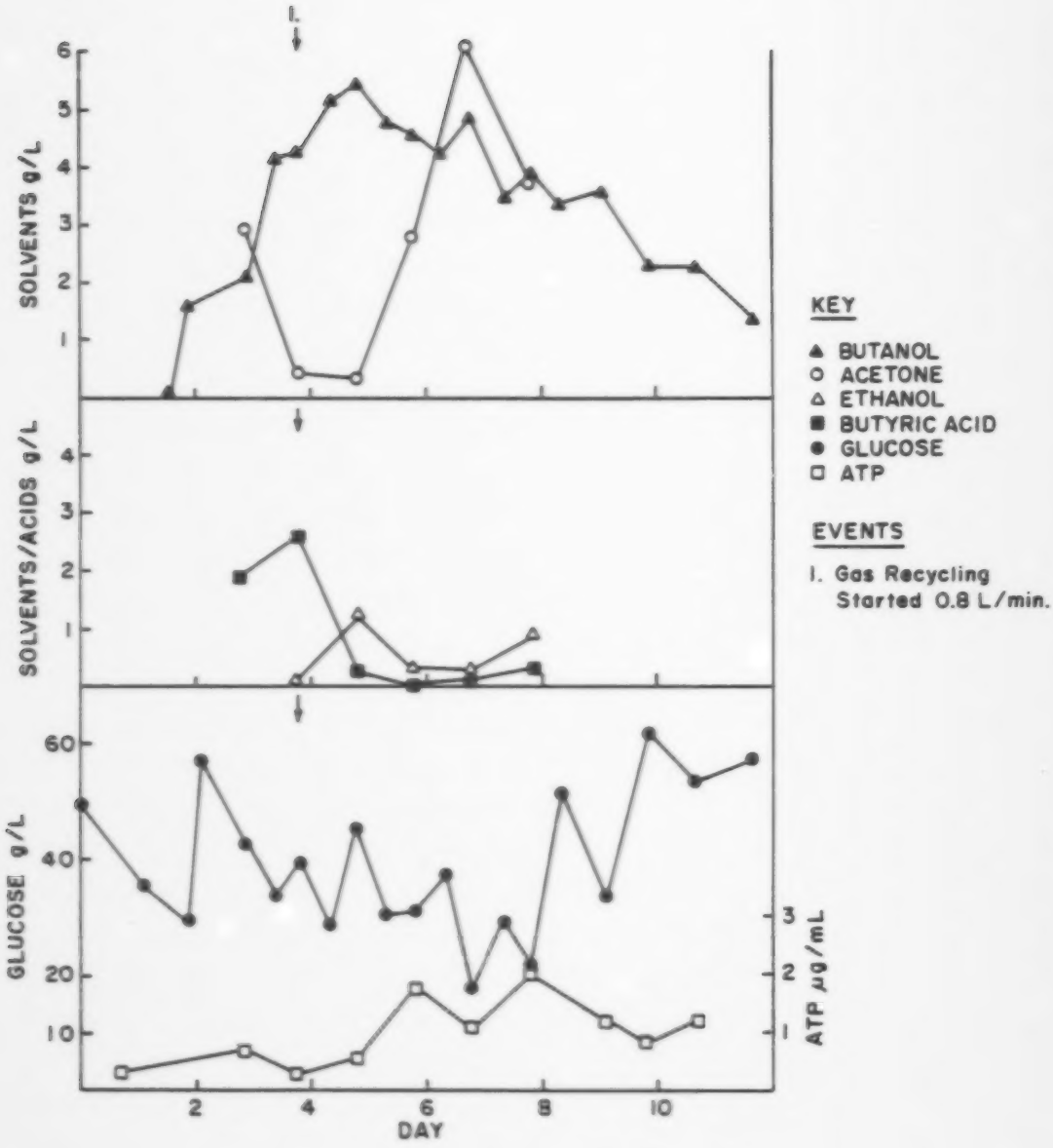

FIGURE 17. FERMENTATION PF2 - FERMENTER-PURIFIER. ANALYSES OF BROTH.

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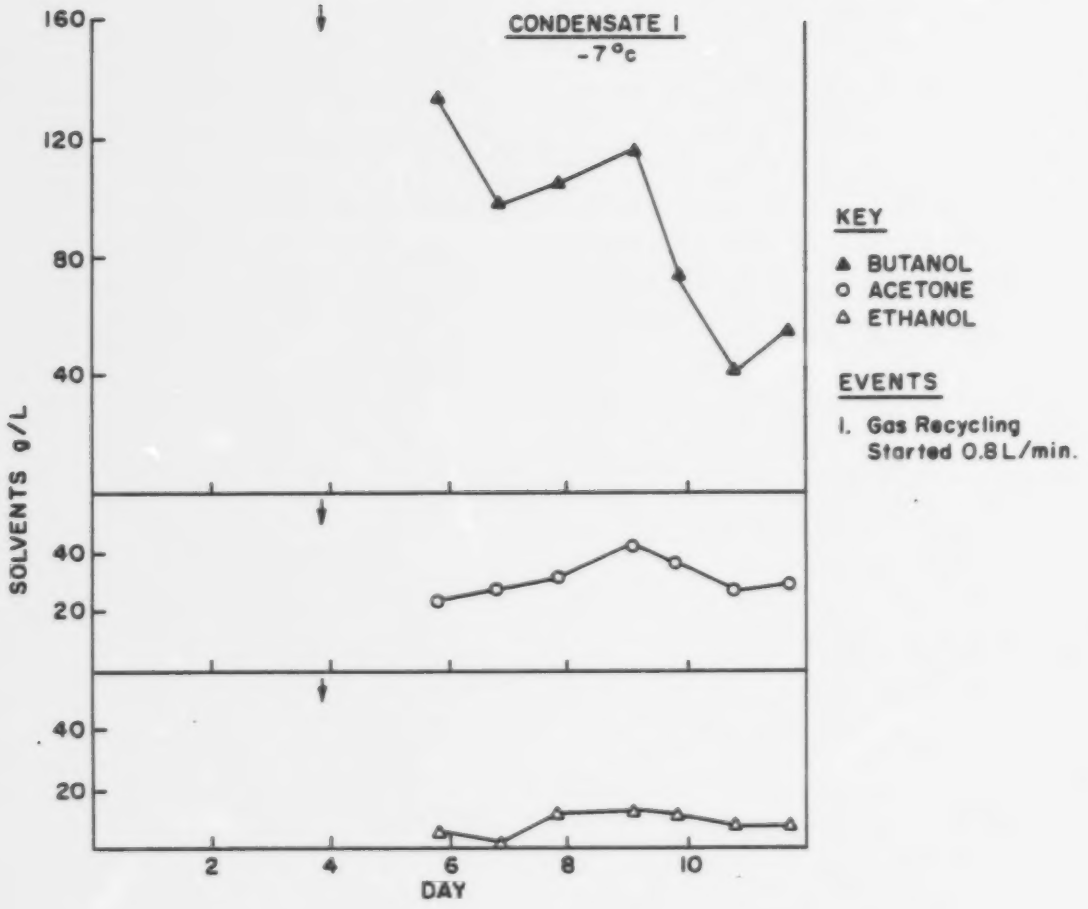

FIGURE 18. FERMENTATION PF2 – FERMENTER-PURIFIER. SOLVENT CONCENTRATIONS IN CONDENSATES.

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TABLE 15: FERMENTATION PF2 - FERMENTER-PURIFIER/MONITORING OF BROTH

DAY	HOUR	pH (1)	ATP ng/mL	GLUCOSE (2)	CONCENTRATION, g/L ACIDS ACETIC	ISO-BUTYRIC	BUTYRIC	CAPROIC	SOLVENTS BUTANOL	ACETONE	ETHANOL	NH_4 mg/L	COMMENTS
0	0												
1	19.0	6.45	300									427	
2	3.0	4.80		35.5									
2	7.5	4.51											
2	21.0	4.27-7.31		29.7					1.55				
3	21.0	5.19	700		ND(3)	0.034	1.89		2.07	2.97	0.001		
4	9.0	5.33		33.9					4.16				
4	19.0		240	39.8	ND	ND	2.55		4.23	0.411		170	Gas recycle started 0.8 L/min.
5	10.0	5.37-4.7		28.9			0.21		5.15				
5	20.0	4.84-4.54	500	45.4	0.07		ND		5.44	0.318	1.164		
6	9.5			30.3					4.70				
6	19.0	4.28-4.80	1750	31.0			0.07		4.52	2.73	0.249		
7	7.5			37.1					4.21				
7	19.0	4.51	1050	17.3					4.85	6.11	0.249	3	Added ammonium sulphate
8	9.0	4.53	470	29.0			0.23		3.49				
8	21.0	4.57	2000	19.6					3.87	3.96	0.870		
9	8.5	4.49-4.73		50.6					3.32				
10	2.5	4.57	1150	33.5					3.56				
10	22.0	4.59	850	61.4					2.26				
11	18.0	4.48	1200	52.9					2.28				Culture contaminated
12	18.0			57.6					1.37				

1. pH before and after adjusting.
2. Glucose concentration before supplementation
3. None detected

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TABLE 16: FERMENTATION PF2 - FERMENTER-PURIFIER/SOLVENT RECOVERY DATA

DAY	CONDENSATE (-7°C) VOLUME (mL)	CONCENTRATION, (g/L) BUTANOL	ACETONE	ETHANOL	SOLVENT RECOVERED (g) BUTANOL	ACETONE	ETHANOL	TOTAL	SOLVENT RATIO	GLUCOSE ADDED (g)
1										24
2										10
3										
4										
5										
6	51	134.0	23.0	4.61	6.834	1.173	0.235	8.242	29:5:1	25
7	31	97.68	27.4		3.028	0.849		3.877		35
8	43.5	105.2	31.94	10.46	4.576	1.389	0.455	6.420	10:3:1	25
9	42.0	115.6	41.86	12.28	4.855	1.758	0.516	7.129	9.5:3.5:1	17.5
10	29.5	73.05	36.81	10.93	2.155	1.086	0.322	3.563	6.75:3.5:1	
11	35.0	41.69	26.3	7.94	1.459	0.921	0.278	2.658	5.25:3.25:1	
12	13.0	55.12	29.6	8.08	0.717	0.385	0.105	1.207	7:3.75:1	
TOTALS	245.0				23.624	7.561	1.911	33.096	12:4:1	136.5

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(b) Solvent Recovery

The condensates obtained from the single condenser (-7°C) followed a similar pattern to those from Condenser #1, in fermentation PF1. As noted previously (PF1), the concentration of acetone and ethanol in the condensates remained relatively constant and did not reflect changes occurring in the respective concentrations of those solvents in the broth. This confirmed previous observations on the low efficiency of condensation of acetone from the recycling vapour phase at -7°C. The butanol concentrations mirrored those of the fermentation broth.

The goal in this test was to extend the period of production of butanol. The solvent recovery data (Table 16) indicate that the production of solvent in PF2 was significantly better than in PF1. Thus 33.10 g of total solvents were recovered within 13 days compared with 41.87 g solvent in 25 days in the case of PF1. The butanol concentration was also higher with the overall mean solvent ratios for butanol/acetone/ethanol being 12:4:1 compared with that of 6:2:1 in PF1. However, although the slower stripping rate appeared to maintain the butanol concentration at a higher level for longer, the production of butanol could not be maintained. It may be seen that although the solvent concentrations were declining, solvents were still being produced in the culture. For example, the data (Table 15) indicate that between Day 8 and 9, 4.855 g butanol were condensed from the vapour phase. The butanol concentration in the broth declined from 3.87 to 3.32 over the same interval. Thus, of the recovered butanol, 0.55 g was attributable to lowering of the broth concentration while the balance (4.305 g) represents the butanol productivity of the culture (1.2 L) over this period.

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(c) Culture Morphology

The culture was examined microscopically at regular intervals. The morphological changes included a transition from short vegetative rods to longer rods often occurring in chains or doublets. At Day 12, the culture was clearly seen to be contaminated. The test was halted at this time.

6.4.7 Fermentation AF2

(a) General Observations

This fermentation in the airlift fermenter (Figure 19, Tables 17 and 18) duplicated test AF1 with silicalite adsorbent being used to recover organic solvents from the recycling vapour phase. However, in this test, the second condenser ($7^{o}C$) was omitted from the circuit and the vapour upon leaving the fermenter passed through the air cooled condenser and direct to the column of adsorbent.

As in the case of AF1, difficulty was experienced due to severe foaming at Day 2 of the fermentation. Although Antifoam 1520 (1% w/v) was added as required to control foaming, it was also necessary to keep the gas flow at 0.05 - 0.075 L/min. to reduce foam generation.

The maximum butanol concentration attained in the broth was only approximately 4.5 g/L, i.e., the start of the gas recycle may have been premature.

The test was continued for 12 days. As was noted in AF1, the solvent (particularly butanol) concentrations declined at a steady rate under the effects of the stripping. However, at Day 12, the concentration of butanol in the broth was still as high as 2 g/L so the test could have continued for a longer period.

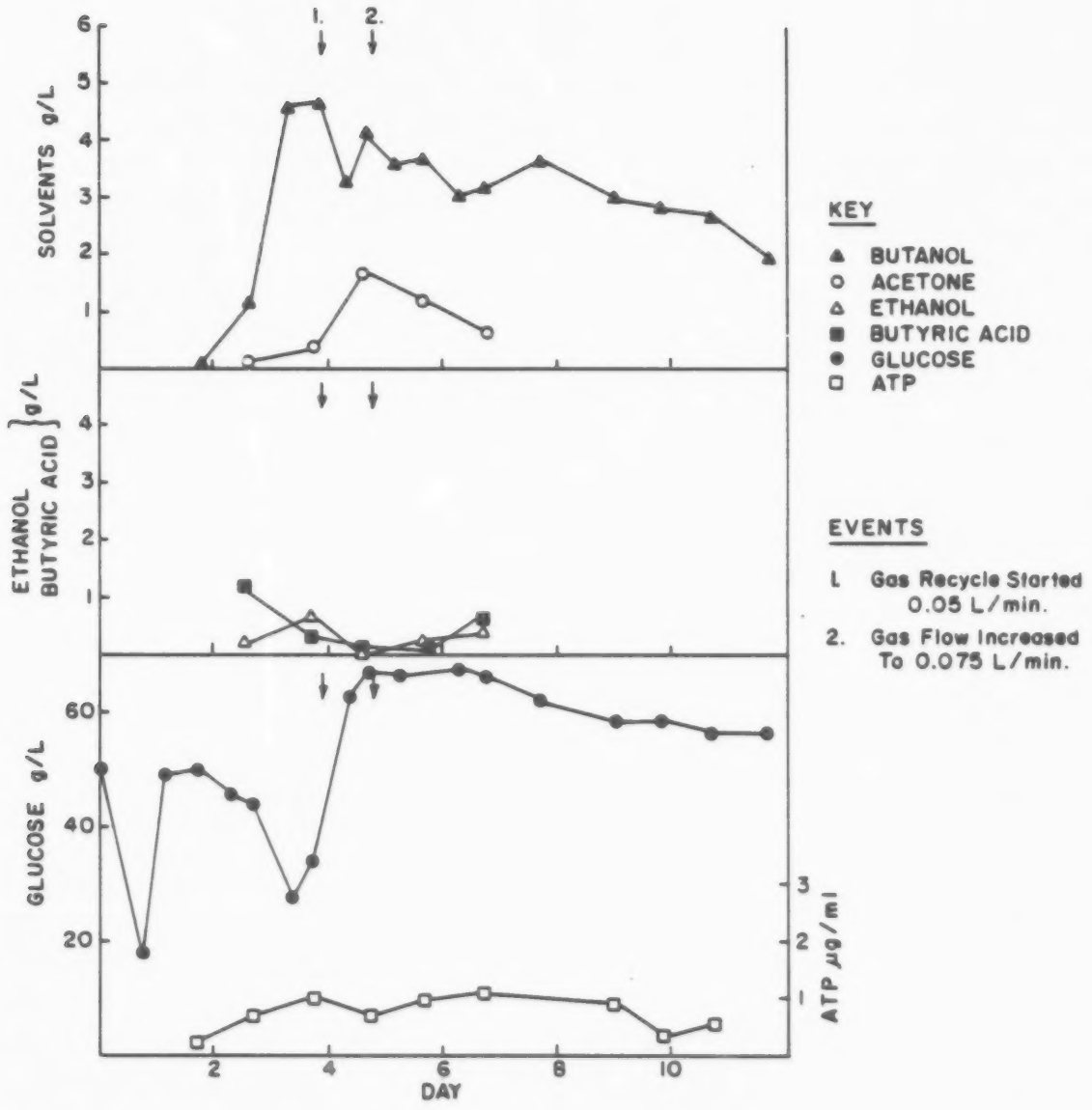

FIGURE 19. FERMENTATION AF2 – AIRLIFT-FERMENTER. ANALYSES OF BROTH.

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TABLE 17: FERMENTATION AF2 - AIRLIFT FERMENTER/MONITORING OF BROTH

DAY	HOUR	pH (1)	ATP ng/mL	GLUCOSE (2)	CONCENTRATION, g/L							NH_4 mg/L	COMMENTS
					ACIDS				SOLVENTS				
					ACETIC	ISO-BUTYRIC	BUTYRIC	CAPROIC	BUTANOL	ACETONE	ETHANOL		
0	0	6.5											
1	18.5	4.05		17.7									
2	3.0	4.80		48.8									
2	18.5	4.60	4.05	49.8									
3	6.5	4.55		45.5									
3	16.0	4.50	700	43.2	0.015		1.155		1.11	0.089	0.176		
4	7.5	4.20		27.4					4.53				
4	17.5	4.30	1000	33.8	0.126		0.283		4.60	0.326	0.644	340	Gas recycle started 0.05 L/min.
5	7.0			62.5					3.30				
5	16.5	4.28	700	66.8	ND(3)		0.142		4.13	1.63	0.100		Increased gas flow rate to 0.075L/min.
6	5.0	4.30		66.43					3.56				
6	16.5	4.52											
		-4.70	950	77.2	ND		ND		3.66	1.16	0.126	160	
7	6.5	4.50		68.8					3.03				
7	18.5	4.30	1100	65.8	ND		0.579		3.15	0.632	0.372		
8	18.0	4.28											
		-4.55		61.8					3.62				
9	0	4.55	875	58.1					3.01				
10	19.5		350	58.4					2.82				
11	15.0		550	56.6					2.69				Culture contaminated

(1) See Table 15
(2) See Table 15
(3) See Table 15

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TABLE 18: FERMENTATION AF2 - AIRLIFT FERMENTER/SOLVENT RECOVERY DATA

DAY	GLUCOSE ADDED	CONDENSATE 1(23°C) VOLUME (mL)	SOLVENTS (g/L) BUTANOL	ACETONE	ETHANOL	CONDENSATE 3A(1) VOLUME (mL)	SOLVENTS (g/L) BUTANOL	ACETONE	ETHANOL	CONDENSATE 3B(2) VOLUME (mL)	SOLVENTS (g/L) BUTANOL	ACETONE	ETHANOL	TOTAL SOLVENTS (g) BUTANOL	ACETONE	ETHANOL	TOTAL
0																	
2	57																
4	50																
5	33																
6						6.9	239.6	12.44	7.319	4.5	137.6	413.2	12.11	2.268	1.945	0.106	4.319
9						8.85	189.8	7.130	6.023	3.8	233.1	267.0	16.08	2.566	1.078	0.114	3.758
12						3.3	40.48			7.0	294.6	107.1	16.0	2.222	0.750	0.112	3.084
13		14	4.257			8.2	110.5	9.537	13.44	2.0	32.09	18.21		1.030	0.114	0.110	1.254
TOTAL	140	14				27.25				17.3				8.086	3.887	0.442	12.415

(1) Solvents desorbed for Silicalite Column and recovered by water cooled condenser (7°C)

(2) Solvent desorbed from Silicalite Column and recovered in liquid nitrogen cooled condenser.

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The acetone concentration peaked approximately 48 hrs. after maximum butanol concentration was attained. However, of note is the fact that as in the case of AF1, the acetone concentration in AF2 was much lower than observed in PF1 and PF2. Thus, it is demonstrated that small changes in culture conditions may change significantly the fermentation pattern and as discussed previously, an inverse relationship may exist between the acetone generation and the production of exopolymer in these cultures, i.e., the viscosity modifier in these cultures.

Glucose consumption was high at the time of maximum solvent (butanol) production but then decreased significantly following this period of high consumption.

As was noted in the previous fermentations, a stable butanol producing fermentation was not established - even with the use of the very low gas recycle rate (0.05 - 0.075 L/min. ≡ 0.027 - 0.042 VVM).

(b) Recovery of Solvents

The silicalite columns effectively recovered solvents from the vapour phase. However, upon thermal desorption, the resultant condensates (Table 18) contained significantly lower butanol concentrations than was observed in fermentation AF1. This is attributed to the fact that water vapour, which in AF1 had been removed predominantly by the condenser cooled with tapwater, was permitted in AF2 to reach the silicalite column. This resulted in condensation of the water on the absorbent due to the temperature differential between the fermenter broth and the adsorbent.

6.5 General Discussion

6.5.1 Yields and Productivity

The data described in the previous sections, verify that butanol/acetone and ethanol can be produced from glucose by the continuous vapour phase stripping of the anaerobic Cl. acetobutylicum fermentation. The overall yield of solvents from each fermentation are shown in Table 19.

In batch fermentations with glucose as substrate, the optimum total solvent yields reported in the literature are in the range 0.31 - 0.34 g solvent/g glucose with production of 0.3 - 0.8 g/L/h (Bahl et al., 1982; Leung and Wang, 1981; Martin et al., 1983). In continuous culture, Bahl et al. (1982) using a two stage, phosphate limited chemostat achieved complete utilization of glucose in order to obtain a total solvent concentration of 18.2 g/L, a yield of 0.34 g/g and a productivity of 0.55 g/L/hr. Bahl et al. used Cl. acetobutylicum DSM 1731, in their studies whereas Leung and Wang (1981) cultured Cl. acetobutylicum ATCC 824 in continuous mode in order to achieve a solvent concentration of 15.9 g/L, a yield of 0.32 g/g and a productivity of 1.5 g/L/hr.

The solvent yields in the fermentations undertaken in this program ranged from 0.074 - 0.089 g/g in the case of the AF1/AF2 fermentations to 0.128 - 0.242 g/g for the PF1/PF2 fermentations. Based on the length of the tests, the respective average productivities were 0.058 g/L/hr. (PF1), 0.088 g/L/hr. (PF2), 0.028 g/L/hr. (AF1) and 0.022 g/L/hr. (AF2).

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TABLE 19: COMPARISON OF DATA FROM FERMENTATIONS

TEST	DESCRIPTION	FERMENTATION VOLUME (L)	NO. OF DAYS	TOTAL CONDENSATE (mL)	TOTAL SOLVENT (g)	MEAN SOLVENT RATIO (CONDENSATE)	OPTIMAL SOLVENT RATIO (CONDENSATES)	ESTIMATED OPTIMAL (1) CONCENTRATION FACTOR FOR BUTANOL	GLUCOSE CONSUMED (g)	SOLVENT YIELD g/g GLUCOSE
PF1	ORF Fermenter-Purifier Condensers -7°C and -16°C	1.2	25	641.2	41.866	6:2:1	31:4.5:1	X30	326.0	0.128
PF2	ORF Fermenter-Purifier Condenser -7°C	1.2	13	245.0	33.096	12:4:1	29:5:1	X27	136.5	0.242
AF1	BRL Airlift Fermenter Condensers 23°C, 7°C and Silicalite	1.8	28	364.0	34.458	13.5:3.5:1	163:15:1	X200 (2)	464.0	0.074
AF2	BRL Airlift Fermenter Condenser 23°C and Silicalite	1.8	13	58.55	12.415	18:9:1	22.5:9.5:1	X57 (2)	140.0	0.089

(1) Based on mean butanol concentration in broth in days preceding condensate collection and analysis

(2) Based on condensate 3A

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On a relative basis, these productivities and yields are much below those of the batch or continuous cultures. However, as stable solvent production was not achieved in any of the fermentations and in view of the complexity of the metabolic processes involved, it is premature to attach too much significance to these data. Subtle changes in environmental and cultural parameters could change significantly, the performance data, i.e. the goal will be to identify and control those factors having greatest influence on solvent production by the organism.

6.5.2 Contamination and Morphological Changes

The lower yields are attributed in part to difficulties experienced with all four fermentations due to the presence of microbial contaminants. It has been recognized during the designing of the system, that contamination of cultures could be a potential problem and attempts were made to achieve a level of aseptic technique such that fermentations could be operated for at least 14 - 21 days. However, this proved to be a troublesome aspect and undoubtedly a significant portion of the glucose added to the fermentations (particularly at the later stages), was consumed by the contaminant species. In view of the decreasing rate of production of solvent, this glucose consumption will have had a negative impact on the calculated yield.

Difficulty was experienced in identifying at precisely which stage of a fermentation did significant numbers of contaminant organisms occur. This was due to diverse morphological changes that Cl. aceto-butylicum is known to undergo when exposed to different cultural conditions, e.g., as fermentations age. Thus, Spivey (1978) identified cell development ranging from long cells, mobile cells, chains of cells, long thin cells with chains common and highly mobile, short cells, club shaped cells, etc. Such observations reflected changes in the cultural characteristics as a laboratory started culture (150 mL) in potato/glucose broth was successfully transferred into finally the plant fermenter (90,000 L) charged with plant bill.

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Early stages of the our fermentations showed the expected pattern with strong populations of short rods, either as singlet or doublets with a high degree of mobility. As the culture aged and particularly when the vapour phase stripping was started, the culture morphology became much more diverse and difficult to interpret. Thus, very long, thin cells were often identified, as well as longer rods of similar diameter to the shorter, mobile rods. These longer cells would often occur as groups of 2 - 3 and mobility was retained in some instances.

As successive fermentations were conducted and our aseptic technique was continuously improved, we became more familiar with the types of changes to be expected. In hindsight, it is possible that several fermentations, which were rejected because of "contamination" may in fact been simply undergoing morphological changes in response to changes in the cultural environment. In view of the variations of culture morphology noted in batch cultures, it is not surprising that the environment created by continuous vapour phase stripping could give rise to more complex mixtures due to factors such as varying solvent concentrations, physiological stress etc.

6.5.3 Solventogenesis in the Fermenter-Purifier

We experienced difficulty in both initiating and maintaining solvent production under conditions of continuous vapour phase stripping. Factors controlling solventogenesis in ABE fermentations are the topic of considerable debate in the literature. Observations of our experiments must be assessed in light of current knowledge and the concept of continuous solvent removal.

A relationship has been purported between the initiation of solventogenesis and the cultural morphology (Jones et al., 1982). Cigar-shaped clostridial forms were implicated in the conversion of organic acids to butanol, acetone and ethanol with the level of solvent production being related to the prevalence of clostridial forms. Thus, solvent product was only detected when a culture was in the stationary growth phase following the production of acids. This concept of solvent production by cells in the stationary phase of growth appears at first glance to match observed cultural behaviour in batch culture. However, in recent years, the attainment of continuous, solvent producing fermentations of Cl. acetobutylicum whose yields match closely those of batch cultures (Bahl et al., 1982; Leung and Wang, 1981; Gottschal and Morris, 1982, Monot and Engasser, 1983) suggests that the morphological changes may reflect changes of the culture environment rather than initiate them. In many instances, these high yielding solventogenic continuous cultures were operated under conditions of either glucose limitation (Wang, 1981), phosphate limitation (Bahl et al. 1982) or nitrogen (ammonia) limitation (Monot and Engasser, 1983). Dilution rates used in these cultures ranged from 0.03 - 0.06, with a lower rate being preferable.

The maintenance of solvent production under conditions of continuous culture does not eliminate the role for clostridial forms in solvent production but does question the concept of the culture being in a stationary phase. Most authors reporting data on continuous culture devote little time to describing the culture morphology and therefore, at the present time, the solution to these possibly conflicting viewpoints remains to be obtained. Of note is the fact, solvent production is apparently greatest when biomass concentration is high (Gottschal and Morris, 1982) hence possibly the highest yields occur at relatively low dilution rates. Indeed, Gottschal and Morris, (1982) maximized their solvent yield by operating their continuous culture as a turbidostat. Thus, culture age would appear to be a factor.

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The relevance of these observations to our fermentations in the fermenter-purifier as operated to date is difficult to assess. In the current tests, the only dilution of the medium occurred when samples were withdrawn for analysis, i.e., a dilution factor of 0.01/day. This is negligible compared with dilution rates in continuous culture of 0.03 - 0.06/hr. Otherwise, the only regular addition to the fermenter-purifier was glucose concentrate to maintain the glucose concentration at the desired level (5% w/v).

When the vapour phase stripping was started in each fermentation, the solvent production was well in progress, hence the cells in the fermenter-purifier culture could be classified as older. The basic hypothesis of the fermenter-purifier concept is that the removal of solvents by gas stripping enables the continued production of solvents in the reactor. To some extent, this goal was achieved although the decreasing rate of solvent production indicates that one or more key parameters effecting the maintenance of the solventogensis have not yet been brought under control and the cells may have undergone transitions to non-solvent producing states.

The loss of solvent production is not surprising since the ABE fermentation with its acidogenic and solventogenic stages is much more complex than, for example, a Saccharomyces cerevisiae fermentation. Thus, while it was noted in the case of S. cerevisiae that ethanol production was maintained albeit at a cyclical rate, in the case of the ABE fermentation maintaining or restoring solventogenesis will depend on establishing the correct cultural/environmental conditions. At the present time, the required operating criteria have still to be identified.

Possible explanations for the tendency of solvent production to decrease steadily following the start of gas recycle and stripping include the following factors. Solvent production in the

fermenter-purifier was initiated best by operating it as a batch fermentation with only a gentle flow of nitrogen gas (0.2 L/min.) to agitate the medium and maintain anaerobic conditions. Prior to starting the gas recycle, the total recycle system was flushed extensively with the nitrogen. The system was then closed and the nitrogen flow stopped. It was anticipated that fermentation gases would help to maintain a positive pressure in the system. This was not the case and while sampling condensates, it was noted that a negative pressure actually was present. While the presence of the slight vacuum indicates the effective sealing of the system, the existence of a vacuum leaves open the possibility of trace amounts of oxygen being sucked into the system. It would require little oxygen in the fermenter to cause a marked reduction in solvent production.

A second factor is the effect of agitation on solvent production. Studies have indicated that agitation reduces the yield of solvents in batch cultures of Cl. acetobutylicum. Subsequently, it was demonstrated (Doremus et al., 1985) that when fermenters were operated under pressure, the deletarious effect of agitation on solvent production was reduced substantially. It is believed that this observation is due to the effect of agitation on the dissolved hydrogen concentration in the fermentation broth. Hydrogen is one of the gaseous by-products of the ABE fermentation and it is hypothesized that in a quiescent culture, the dissolved hydrogen concentration approaches saturation which leads to feedback inhibition of the hydrogen producing pathway. This in turn drives the cellular metabolism towards solvent production. Under conditions of agitation, the dissolved hydrogen concentration is reduced, leading to increased production of hydrogen and the reduced production of solvents. In both small scale bench tests and tests in the fermenter-purifier system, the use of agitation from the time of inoculation, e.g., gas stripping of the broth with either recycled fermenter gases or nitrogen gave rise to poor or no production of

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solvents. Therefore, there is a strong probability that the initiation of gas recycle in an active solvent producing culture may also have led to loss of solvent production due to the disturbance of the dissolved hydrogen concentration.

The maintenance of solventogenesis in Cl. acetobutylicum cultures by the continuous removal of butanol and other solvents by extractive fermentation was the goal of a related study (Taya et al., 1985). These authors, using oleyl alcohol as extractant for butanol maintained butanol production in a fed-batch culture for at least 8 days. Although the rate of fermentation subsequently declined due, it was believed, to the accumulation of acetone and acetic acid in the broth, the data indicated that the continuous fermentation/removal of butanol is feasible. In the Taye system, the oleyl alcohol was present as a layer on the top of the fermentation broth. Although agitation was used (100 rpm), it is conceivable that the presence of organic layer helped to maintain both an anaerobic environment and a dissolved hydrogen concentration in the broth.

The other factors which may lead to a loss of solventogenesis may be nutrient imbalance or the accumulation of inhibitory compounds in the fermenter-purifier. Butyric and acetic acids are very toxic to the organism but there was no indication in the fermentation that the concentrations at any time reached critical threshold levels. Depletion of the dissolved ammonia concentration was noted but supplements failed to maintain or restore butanol production.

It is apparent that much has still to be learned of the metabolic factors controlling the initiation and maintenance of solventogenesis in Cl. acetobutylicum cultures under the conditions of continuous recovery of solvents by vapour phase stripping. Factors which appear to be significant for continuous production of solvents include

the nutrient status of a culture, parameters with potential feedback effects, e.g., hydrogen, carbon dioxide partial pressures, pH, organic acid concentrations, etc. Additional studies are required to optimize those parameters relevant to the operating characteristics of the fermenter-purifier concept. This will require the design of a more gas tight system so that both the maintenance of anaerobiosis can be guaranteed and the partial pressures of gaseous by-products (hydrogen, carbon dioxide) can be controlled and measured. Furthermore, the system must be capable of more efficient sterilization to ensure freedom from contamination.

At the present time, the significance of the temperature on the solvent yields and proportions of the individual solvents cannot be interpreted. In these tests, inocula developed from largely frozen vegetative cultures were used. Heat-shocking may be required to afford better solvent producing cultures.

6.6 Condensation

The efficiency with which the respective solvents are removed from the recycling vapour phase will influence the concentrations of the respective solvents in the fermentation broths. At no time in the fermentations described herein did the solvent concentrations reach measured levels that might be considered toxic. The observation in PF1 and PF2 that the acetone and ethanol concentrations in the broths of PF1 and PF2 tended to reach a maximum several days after the butanol concentration had peaked but that a similar pattern was not observed in AF1 and AF2 warrants examination. The key differences between the two systems were (1) that the AF1 and AF2 fermentations used significantly slower gas recycle rates than in the case of the fermenter-purifier (PF) tests, and, (2) the condenser systems for solvent recovery were different. The removal of solvent (particularly butanol) was more rapid

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in the PF system and the acetone accumulation may reflect a metabolic transition, following the initiation of the gas recycle. A related aspect is that another gaseous by-product, the carbon dioxide, arises in part as the metabolic intermediate acetoacetyl co-enzyme A is converted to acetone (Haggstrom, 1985). It is conceivable that the reduction of the dissolved carbon dioxide concentration in the broth due to the effect of the gas stripping resulted in an acceleration, albeit, short-lived, in the acetoacetyl conversion to acetone and carbon dioxide, as a feedback inhibition effect was removed. The lower gas recycle rate in the AF system would not have disturbed the concentrations of dissolved gases to the same extent hence the rise in the acetone concentration would be less noticeable.

Another factor is the relative efficiencies of the different condenser system to remove acetone from the vapour phase. As noticed from the compositions of the condensates from PF1 and PF2, the efficiency of the acetone condensation was poor at $-7^{o}C$ and $-16^{o}C$ respectively and did not mirror the respective concentrations in the broth. Silicalite is believed to be a more effective method to remove acetone from the vapour phase and the lower levels measured in the AF1 and AF2 broths may reflect this fact.

The use of the hydrophobic molecular sieve adsorbent to recover ethanol from the vapour phase has been patented (Garg and Ausikaitis, 1985). Their process referred to the vapour phase stripping of fermented beers. However, the inclusion of the silicalite or similar types of hydrophobic molecular sieve as an integral part of the fermenter-purifier concept warrants further attention. In the tests as conducted, it is apparent that the efficiency of the thermal desorption of adsorbed organic compounds must be improved and more data are required on the adsorption isotherms of the target compounds.

The indication is that the butanol will be preferentially adsorbed but that a partial fractionation of the butanol from the acetone and ethanol can be achieved by both control of the desorption temperature and the appropriate design of the condenser system. A preliminary low temperature desorption stage will remove most of the condensed water (Maddox, 1983) and result in more concentrated butanol solutions being recovered from the adsorbent. Alternatively, a process schematic could incorporate a $-7^{o}C$ condenser to remove much of the butanol followed by a silicalite condenser to recover preferentially the acetone and ethanol.

6.7 Economic Aspects

The inability to maintain stable, high yielding solvent producing cultures of Cl. acetobutylicum in the fermenter-purifier at the present time means that an approperiate mass balance and economic assessment cannot be undertaken. However, the results from these exploratory studies indicate that many potential economic advantages may accrue from the application of the fermenter-purifier concept to the Cl. acetobutylicum fermentation. Phillips and Humphrey (1983) showed that at butanol concentrations less than 3 - 4 % by weight, the net energy recovery and hence the economics are poor. Accordingly, the ability of the fermenter- purifier to afford condensates as high as 9 - 12% by weight with a condenser at $-7^{o}C$ is a significant improvement. At this concentration, the energy requirements for further purification of the butanol are close to the minimal level. Furthermore, the generation of butanol solutions in excess of 50% by weight butanol by the use of molecular sieve adsorbents enhances still further the attractiveness of the fermenter-purifier concept.

Lenz and Moreira (1980) undertook an economic analysis for a 45 x 10^{6} Kg solvents/year plant based on molasses as feedstock. They concluded that the process economics were very unfavourable compared with

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petro- chemical-based processes. This was based on the traditional batch fermentation concept and required a large number of fermenters (16 x 15.4 m^3) as well as a significant solvent recovery system. Furthermore, a high volume of stillage had to be processed e.g., 5 evaporators were needed. The fermenter-purifier concept offers potential solutions and/or improvements to each of these areas and would result in significant reductions in the capital costs for a plant, since the above factors represented approximately 82% of the equipment costs.

More significantly, the feedstock (molasses) represented approximately 63% of the total production costs, hence economic feasibility will depend to a large extent on the solvent yield obtained. The fermenter- purifier design will allow significant manipulation of cultural factors to achieve improved yields once the solvent production rate can be stabilized.

Lenz and Moreira identified liquid whey as a highly suitable and much less expensive feedstock. This substrate would be suited to the fermenter-purifier system. In view of the potential to reduce significantly both the fermenter capacity that would be needed and the energy intensive distillation recovery and stillage processing systems for such a plant, the fermenter-purifier should be able to improve significantly on the attractiveness of the process economics of a whey-based plant.

7. CONCLUSIONS AND RECOMMENDATIONS

The current tests have verified that the concept of vapour phase stripping of butanol, acetone and ethanol from dilute fermentation broths is feasible.

However, the ABE fermentation is complex with a great deal still to be learned as to the mechanism for initiation and maintenance of solvent production. Accordingly, although long term continuous fermentation was not established in the current program, it is believed that the potential for further development is high.

It is recommended that future studies concentrate on several key areas, namely;

1. Design of a fully sterilizable, stainless steel system capable of operating under positive pressure.

2. Tests with this system to monitor and control with greater precision, gas product ratios, partial pressures, nutrient feeds.

3. Tests to assess the effect of nutrient composition, e.g., glucose limitation, phosphate limitation, ammonia limitation on the maintenance of solvent producing cultures.

4. Tests for role of secondary nutrients, e.g., ferrous iron, vitamins, etc. - possibly satisfied by semi-continuous operation.

5. Optimization of the use of solid phase molecular sieve adsorbents for recovery of volatile products from the vapour phase.

6. Evaluation of high-efficiency condenser systems.

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APPENDIX I

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APPENDIX II

BRL Airlift Fermenter

Components of the BRL Airlift Fermenter

Case

The seamless, wipe-clean case is made of chemical- and temperature-resistant ABS, specially formulated for BRL. Within the case are housed the other components: reactor vessel, built-in lights, and control console.

Control Console

The console consists of five independent, precision-calibrated control panels, each with its own circuit breaker. These panels regulate the five environmental control systems.

pH monitor/control system: The BRL Airlift Fermenter is provided with a steam-sterilizable combination pH electrode and a pH meter which also serves as a single-limit controller. When any preset pH limit (either high or low) is exceeded, the pH output is energized at 110 volts and 300 watts, which is sufficient to operate an alarm, peristaltic pump or a solenoid valve.

Foam/liquid level control system: This system, consisting of an electronic alarm, a conductivity-type controller and an AC output, can be used to detect either an insufficient liquid level due to evaporation or excessive media foaming. In either case, a pulsating audio alarm is activated, and power becomes available at the AC output. If desired, a peristaltic pump can be connected to the output to correct the foam or liquid level by adding an antifoam reagent or sterile water. In continuous culture mode, the pump also can be used to add media.

Temperature control system: The temperature system consists of a 350 watt stainless steel immersion heater, a type J thermocouple, and an electronic control for temperatures ranging from 0 to 121°C. A stainless steel cooling probe also is provided for cooling the vessel more rapidly from sterilization temperatures. The cooling probe can be used with an ice reservoir and pump, a water chiller, or a single-pass hookup between tap water and return to drain. The wide range of temperatures that can be attained by the BRL Airlift Fermenter makes it suitable for growing both thermophiles and psychrophiles, as well as those thriving in more temperate environments.

Air/gas mixing system: In addition to two gas entry ports which provide the capability to custom-mix any two gases (or gas and air) supplied by the user, the BRL Airlift Fermenter also has a built-in air compressor, eliminating the need for house air. The fermenter's air pressure adjusting capability helps maintain the sterility of the vessel and enhances mass transfer rates.

Reactor vessel

The 1.8 liter (working volume) vessel is constructed of heavy-wall, type I class A borosilicate glass, with an outer diameter of 3½ inches. It is divided vertically by a glass baffle plate, offset from the center such that the volumes of the upflow and downflow regions are approximately 67% and 33%, respectively. This ratio increases the fluid velocity in the downflow region relative to the upflow velocity under a constant relative pressure differential, contributing to efficient mass transfer.

Air is delivered into the bottom of the upflow region through a 316 stainless steel sparger. The sparger's 0.5 micron pore diameter prevents microbial fouling. The top of the vessel also contains a zero-dead-volume sampling port, as well as five ports to facilitate the introduction of probes or solid additives into the growth medium.

Fully-controllable, built-in lights

A bank of five cold cathode lights illuminates the vessel. These broad-spectrum (daylight) lamps are included as standard equipment, and other specific wavelength windows are available as options.

Illumination control system: Designed specifically for culturing photosynthetic organisms, the light system includes a sensitive photovoltaic cell which indicates the light levels on the inside wall of the fermenter vessel. The flux density of the five lamps is fully controllable from 0 to 400 μE m⁻² sec⁻¹.

Components of the BRL Airlift Fermenter

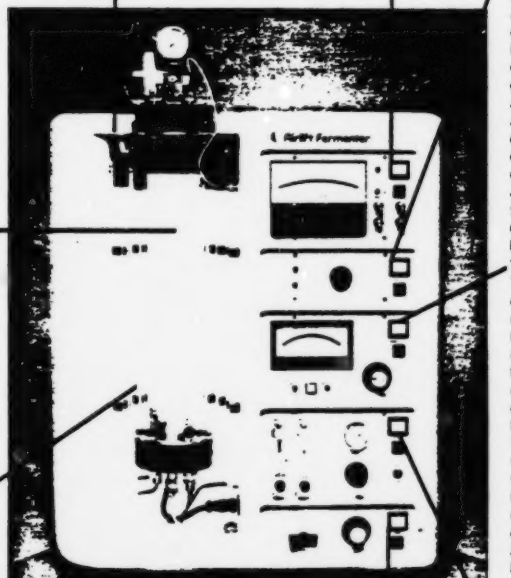
Case

The seamless, vapor-tight case is made of chemical- and temperature-resistant ABS specially formulated for BRL. Within the case are housed the other components: reactor vessel, built-in lights, and control console.

Control Console

The console consists of five independent, precision-calibrated control panels, each with its own circuit breaker. These panels regulate the five environmental control systems.

pH monitor/control system: The BRL Airlift Fermenter is provided with a steam-sterilizable combination pH electrode and a pH meter which also serves as a single-limit controller. When any preset pH limit (either high or low) is exceeded, the pH output is energized at 110 volts and 300 watts, which is sufficient to operate an alarm, peristaltic pump or a solenoid valve.



Reactor vessel

The 1.8 liter (working volume) vessel is constructed of heavy-wall, type I, class A borosilicate glass, with an outer diameter of 3½ inches. It is divided vertically by a glass baffle plate, offset from the center such that the volumes of the upflow and downflow regions are approximately 67% and 33%, respectively. This ratio increases the fluid velocity in the downflow region relative to the upflow velocity under a constant relative pressure differential, contributing to efficient mass transfer.

Air is delivered into the bottom of the upflow region through a 316 stainless steel sparger. The sparger's 0.5 micron pore diameter prevents microbial fouling. The top of the vessel also contains a zero-dead-volume sampling port, as well as five ports to facilitate the introduction of probes or solid additives into the growth medium.

Fully-controllable, built-in lights

A bank of five cold cathode lights illuminates the vessel. These broad-spectrum (daylight) lamps are included as standard equipment, and other specific wavelength windows are available as options.

Foam/liquid level control system: This system, consisting of an electronic alarm, a conductivity-type controller and an AC output, can be used to detect either an insufficient liquid level due to evaporation or excessive media foaming. In either case, a pulsating audio alarm is activated and power becomes available at the AC output. If desired, a peristaltic pump can be connected to the output to correct the foam or liquid level by adding an antifoam reagent or sterile water. In continuous culture mode, the pump also can be used to add media.

Temperature control system: The temperature system consists of a 350 watt stainless steel immersion heater, a type J thermocouple, and an electronic control for temperatures ranging from 0 to 121°C. A stainless steel cooling probe also is provided for cooling the vessel more rapidly from sterilization temperatures. The cooling probe can be used with an ice reservoir and pump, a water chiller, or a single-pass hookup between tap water and return to drain. The wide range of temperatures that can be attained by the BRL Airlift Fermenter makes it suitable for growing both thermophiles and psychrophiles, as well as those thriving in more temperate environments.

Illumination control system: Designed specifically for culturing photosynthetic organisms, the light system includes a sensitive photovoltaic cell which indicates the light levels on the inside wall of the fermentation vessel. The flux levels of the five lamps is fully controllable from 0 to 400 μE m⁻² sec⁻¹.

Air/gas mixing system: In addition to two gas entry ports which provide the capability to custom-mix any two gases (or gas and air) supplied by the user, the BRL Airlift Fermenter also has a built-in air compressor, eliminating the need for house air. The fermenter's air pressure adjusting capability helps maintain the sterility of the vessel and enhances mass transfer rates.

SPECIFICATIONS

REACTOR VESSEL

Nominal working volume: 1800 cc
Column, baffle and welds constructed of heavy wall type 1 Class A borosilicate glass (identical to Kimble KG33, Corning 7740), nominal 3.5" O.D. x 3.12" I.D. x 3/16" wall
All other wetted parts: 316 stainless steel, black polypropylene, Viton fluoroelastomer

pH CONTROL SYSTEM

pH range: 0-14, high or low limit set point
Temperature compensation: 0-100°C
Indication: six inch meter with mirror back scale providing 2% FS accuracy
Power output: up to 1/4 amp at 110 volts

ELECTRODE

Steam-sterilizable combination electrode

HEATER, TEMPERATURE CONTROL

"Push to Set" calibration
Operating range: 0-121°C
Type J thermocouple
Heater output to 350 watts
Control accuracy to less than 0.5°C.
Automatic cold junction compensator
Solid state relay

AIR/GAS DELIVERY SYSTEM

Compressor

100% oil-free operation
Contamination-free pumping of air, gas or vapor
Luran/Viton construction
Flow capacity: 9.2 liters/min
Maximum continuous pressure: 7 psi

Flow Meters

Dwyer VF Series custom flow meters
316 stainless steel metering valves
Each unit individualy calibrated
Precision bored to $\pm$ 5% accuracy

Connections

All contactable fittings, 316 stainless steel
"Kwik Connect" fittings on bulkhead vessel

Sparger

316 stainless composition
0.5 micron pore size

FOAM/LEVEL CONTROL

Conductivity principle controller
Wetted parts: 316 stainless and Teflon®
3/4 second time delay to eliminate "chirps"
Power output to 1/4 amp at 110 volts
Sensing current alternates at 7KHz to prevent deterioration of sensor tips and electrolytic product contamination

ILLUMINATION SYSTEM

Five mercury vapor lamps, 13 mm diameter x 44" overall length, "U" configuration
Console-controlled light level, fully dimmable
Console-mounted flux density meter reading $\mu E/m^2$ sec with Solarex sensor
Wavelength changeable by lampset
Output range (Daylight White) 0-300 $\mu E/m^2$ sec

CONSOLE

Overall: 25" W x 17" D x 36" H (64 cm x 44 cm x 91 cm)
Construction: high temperature-resistant molded ABS
Weight, including reactor vessel: 65 lbs (29.5 kg)
Power requirements: 110 volts AC

ACCESSORY PACKAGE

Rugged, latched copolymer tool case
Vessel and manifold cleaning brushes
1000" Teflon threadseal/doping tape
3 feet pneumatic tubing
Spare Gelman membrane filter
Spare resealable injection septa
Filling funnel

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